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Date: June 16, 2000

Docket No.: 2750-942P

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

As authorized by the inventor(s), transmitted herewith for filing is a patent application applied for on behalf of the inventor(s) according to the provisions of 37 C.F.R. § 1.41(c), which claims priority under 35 U.S.C. § 119(e) of Provisional Application No. 60/139,763 filed on June 18, 1999

Inventor(s): Nickolai ALEXANDROV, Maxim TROUKHAN

For: SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

Enclosed are:

- ☒ A specification consisting of a description (866 pages), Table 1 (725 pages), Claims (5 pages), Schematic 1 (1 page), and Abstract (1 page) totaling one thousand five hundred ninety-eight (1598) pages
- ☐ ( ) sheet(s) of formal drawings
- ☐ Certified copy of Priority Document(s)
- ☒ Executed Declaration in accordance with 37 C.F.R. § 1.64 will follow
- ☒ A statement to establish small entity status under 37 C.F.R. § 1.9 and 37 C.F.R. § 1.27

☐ Preliminary Amendment

☒ Information Sheet

☐ Information Disclosure Statement, PTO-1449 and reference(s)

☐ Amend the specification by inserting before the first line the sentence:

--This application claims priority on provisional Application No. filed on , the entire contents of which are hereby incorporated by reference.--

☒ Other: Power of Attorney regarding Small Entity Statement, ATCC deposit receipts PTA-595, PTA-1161, PTA-1411, CD containing specification.

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The filing fee has been calculated as shown below:

			LARGE ENTITY	**SMALL ENTITY**
	BASIC FEE		**$690.00**	**$345.00**
	NUMBER FILED	NUMBER EXTRA	RATE FEE	RATE FEE
TOTAL CLAIMS	50- 20 =	30	X 18 = $0.00	x 9 = $270.00
INDEPENDENT CLAIMS	5- 3 =	2	x 78 = $0.00	x 39 = $78.00
☐ MULTIPLE DEPENDENT CLAIMS PRESENTED			+ $260.00	+ $130.00
		TOTAL	$0.00	$693.00

☒ The application transmitted herewith is filed in accordance with 37 C.F.R. § 1.41(c). The undersigned has been authorized by the inventor(s) to file the present application. The original duly executed declaration together with the surcharge will be forwarded in due course.

☒ A check in the amount of $693.00 to cover the filing fee is enclosed.

☐ Please charge Deposit Account No. 02-2448 in the amount of $0.00. A triplicate copy of this transmittal form is enclosed.

☒ Please send correspondence to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP **or** Customer No. 2292
P.O. Box 747
Falls Church, VA 22040-0747
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If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By #36,623
Raymond C. Stewart, #21,066

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RCS/DRN/JDK
2750-942P

Attachments

(Rev. 04/19/2000)

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PATENT
2750-942P

IN THE U.S. PATENT AND TRADEMARK OFFICE

INFORMATION SHEET

Applicant:	Nickolai ALEXANDROV, Maxim TROUKHAN
Appl. No.:	NEW
Filed:	June 16, 2000
For:	SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

Priority Claimed: 2750-465P 60/139,763 June 18, 1999

09595326.061600

Send Correspondence to:

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The above information is submitted to advise the U.S.P.T.O. of all relevant facts in connection with the present application.

A timely executed Declaration in accordance with 37 C.F.R. § 1.64 will follow.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

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RCS/DRN/JDK
2750-942P

(Rev. 04/19/2000)

STATEMENT CLAIMING SMALL ENTITY STATUS Docket Number: 2750-942P
(37 CFR 1.9(f) & 1.27(c)) - SMALL BUSINESS CONCERN

Applicant, Patentee, or Identifier: Nickolai ALEXANDROV, Maxim TROUKHAN
Application or Patent No.: NEW Patent Application
Filed or Issued: June 16, 2000
Title: SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

I hereby state that I am
☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN CERES, INC.
ADDRESS OF SMALL BUSINESS CONCERN 3007 Malibu Canyon Road Malibu, CA 90265

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 37 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

☒ the specification filed herewith with title as listed above.
☐ the application identified above.
☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:
☒ no such person, concern, or organization exists.
☐ each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is not longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING Mark J. Nuell (Reg. No. 36, 623)

TITLE IN ORGANIZATION OF PERSON SIGNING Legal Representative of CERES, INC.

ADDRESS OF PERSON SIGNING Birch, Stewart, Kolasch and Birch, LLP.

P.O. Box 747 Falls Church, VA 22040-0747

SIGNATURE MJ Nuell DATE June 16, 2000

Rev. 10/12/1998

SEQUENCE-DETERMINED DNA FRAGMENTS AND CORRESPONDING POLYPEPTIDES ENCODED THEREBY

This application claims priority under 35 USC §119(e), §119(a-d) and §120 of the following applications, the entire contents of which are hereby incorporated by reference:

Country	Filing Date	Attorney No.	Client No.	Application No.
United States	06/18/99	2750-0465P	00038.001	60/139,763

FIELD OF THE INVENTION

The present invention relates to isolated polynucleotides that represent a complete gene, or a fragment thereof, that is expressed. In addition, the present invention relates to the polypeptide or protein corresponding to the coding sequence of these polynucleotides. The present invention also relates to isolated polynucleotides that represent regulatory regions of genes. The present invention also relates to isolated polynucleotides that represent untranslated regions of genes. The present invention further relates to the use of these isolated polynucleotides and polypeptides and proteins.

DESCRIPTION OF THE RELATED ART

Efforts to map and sequence the genome of a number of organisms are in progress; a few complete genome sequences, for example those of *E. coli* and *Saccharomyces cerevisiae* are known (Blattner et al., *Science* 277:1453 (1997); Goffeau et al., *Science* 274:546 (1996)). The complete genome of a multicellular organism, *C. elegans*, has also been sequenced (See, the *C. elegans* Sequencing Consortium, *Science* 282:2012 (1998)). To date, no complete genome of a plant has been sequenced, nor has a complete cDNA complement of any plant been sequenced.

SUMMARY OF THE INVENTION

The present invention comprises polynucleotides, such as complete cDNA sequences and/or sequences of genomic DNA encompassing complete genes, fragments of genes, and/or regulatory elements of genes and/or regions with other functions and/or intergenic regions, hereinafter collectively referred to as Sequence-Determined DNA Fragments (SDFs), from different plant species, particularly corn, wheat, soybean, rice and *Arabidopsis thaliana,* and other plants and or mutants, variants, fragments or fusions of said SDFs and polypeptides or proteins derived therefrom. In some instances, the SDFs span the entirety of a protein-coding segment. In some instances, the entirety of an mRNA is represented. Other objects of the invention that are also represented by SDFs of the invention are control sequences, such as, but not limited to, promoters. Complements of any sequence of the invention are also considered part of the invention.

Other objects of the invention are polynucleotides comprising exon sequences, polynucleotides comprising intron sequences, polynucleotides comprising introns together with exons, intron/exon junction sequences, 5' untranslated sequences, and 3' untranslated sequences of the SDFs of the present invention. Polynucleotides representing the joinder of any exons described herein, in any arrangement, for example, to produce a sequence encoding any desirable amino acid sequence are within the scope of the invention.

The present invention also resides in probes useful for isolating and identifying nucleic acids that hybridize to an SDF of the invention. The probes can be of any length, but more typically are 12-2000 nucleotides in length; more typically, 15 to 200 nucleotides long; even more typically, 18 to 100 nucleotides long.

Yet another object of the invention is a method of isolating and/or identifying nucleic acids using the following steps:

(a) contacting a probe of the instant invention with a polynucleotide sample under conditions that permit hybridization and formation of a polynucleotide duplex; and

(b) detecting and/or isolating the duplex of step (a).

The conditions for hybridization can be from low to moderate to high stringency conditions. The sample can include a polynucleotide having a sequence unique in a plant genome. Probes and methods of the invention are useful, for example, without limitation, for mapping of genetic traits and/or for positional cloning of a desired fragment of genomic DNA.

Probes and methods of the invention can also be used for detecting alternatively spliced messages within a species. Probes and methods of the invention can further be used to detect or isolate related genes in other plant species using genomic DNA (gDNA) and/or cDNA libraries. In some instances, especially when longer probes and low to moderate stringency hybridization conditions are used; the probe will hybridize to a plurality of cDNA and/or gDNA sequences of a plant. This approach is useful for isolating representatives of gene families which are identifiable by possession of a common functional domain in the gene product or which have common cis-acting regulatory sequences. This approach is also useful for identifying orthologous genes from other organisms.

The present invention also resides in constructs for modulating the expression of the genes comprised of all or a fragment of an SDF. The constructs comprise all or a fragment of the expressed SDF, or of a complementary sequence. Examples of constructs include ribozymes comprising RNA encoded by an SDF or by a sequence complementary thereto, antisense constructs, constructs comprising coding regions or parts thereof, constructs comprising promoters, introns, untranslated regions, scaffold attachment regions, methylating regions, enhancing or reducing regions, DNA and chromatin conformation modifying sequences, etc. Such constructs can be constructed using viral, plasmid, bacterial artificial chromosomes (BACs), plasmid artificial chromosomes (PACs), autonomous plant plasmids, plant artificial chromosomes or other types of vectors and exist in the plant as autonomous replicating sequences or as DNA integrated into the genome. When inserted into a host cell the construct is, preferably, functionally integrated with, or operatively linked to, a heterologous polynucleotide. For instance, a coding region from an SDF might be operably linked to a promoter that is functional in a plant.

The present invention also resides in host cells, including bacterial or yeast cells or plant cells, and plants that harbor constructs such as described above. Another aspect of the invention relates to methods for modulating expression of specific genes in plants by expression of the coding sequence of the constructs, by regulation of expression of one or more endogenous genes in a plant or by suppression of expression of the polynucleotides of the invention in a plant. Methods of modulation of gene expression include without limitation (1) inserting into a host cell additional copies of a polynucleotide comprising a coding sequence; (2) modulating an endogenous promoter in a host cell; (3) inserting antisense or ribozyme constructs into a host cell and (4) inserting into a host cell a polynucleotide comprising a sequence encoding a variant , fragment, or fusion of the native polypeptides of the instant invention.

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BRIEF DESCRIPTION OF THE TABLES

In TABLE 1, the format of the data is as follows:

In Table 1, sequence data are presented in the form of annotation of a reference sequence. The format is shown below. The reference sequence is shown at the top of the annotation file as a 7 digit sequence number preceded by “>” (*e.g.* >5019261). The sequence identifier is a “gi” number that identifies a specific DNA sequence in the publically accessible BLAST Databases on the NCBI FTP web site (accessible at ncbi.nlm.gov/blast). In particular, the “nt.Z” nucleotide sequence data base at the NCBI FTP site utilizes the “gi” identifiers to assign by NCBI a unique identifier for each sequence in the databases, thereby providing a non-redundant database for sequences from various data bases, including

GenBank, EMBL, DDBJ (DNA Database of Japan) and PDB (Brookhaven Protein Data Bank). Thus, the line in TABLE 1 beginning with sequence number identifies the unique “gi” identifier followed by the corresponding GenBank (gb) accession number and locus. The reference sequence number is followed on the next line by data regarding the length of the sequence (“len”) and the number of exons found in the sequence by the analysis program (“nex”).

The annotation data are presented in columns; the leftmost column identifies the position of the putative exon in the gene as initial (“init”), internal (“intr”) or terminal (“term”). Genes considered composed of a single exon are denoted “sngl”. The next column describes the position in the nucleotide sequence beginning the exon (“start”) and the next column describes the position in the nucleotide sequence ending the exon (“stop”). The direction of the gene is indicated in the next column, “+” indicating 5’ - 3’ in the direction presented in the database, “-“ indicating the opposite orientation. The “gene number” is given in the final column. Exons having the same gene number are grouped in the order shown to create the relevant coding sequence.

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>5019261 ⇐ This is the gi number of the public sequence

len = 97208 nex = 121

⇑ ⇑

Length of public sequence — Number exons

Exon Type ⇓	Start ⇓	Stop ⇓	Direction ⇓	Gene Number ⇓
Sngl	602	778	+	0
Sngl	990	1316	+	1
Sngl	2356	2691	+	2
Sngl	4634	4735	+	3
Sngl	4973	5092	+	4
Sngl	5746	5874	+	5
Init	8119	8798	+	6
Term	9284	9518	+	6
Init	10827	11150	+	7
Term	11294	11335	+	7
Sngl	12655	12825	+	8
Sngl	13303	13596	+	9
Sngl	18654	18782	+	10
Sngl	19880	20086	+	11
Init	21476	21539	+	12
Intr	21647	21802	+	12
Term	23488	23567	+	12
Init	25035	25133	+	13
Intr	25466	25589	+	13
Intr	25677	25786	+	13
Intr	25899	25962	+	13
Intr	26045	26109	+	13
Intr	26188	26253	+	13
Term	26350	26448	+	13
Sngl	27671	27793	+	14
Sngl	29126	29299	+	15
Sngl	30266	30364	+	16
Sngl	31717	31929	+	17
Sngl	32102	32209	+	18
Sngl	32450	32548	+	19

Sngl	32634	32726	+	20
Init	35603	35743	+	21
Term	35829	36185	+	21
Init	36954	37098	+	22
Term	38100	38158	+	22
Init	39635	39944	+	23
Intr	40242	40372	+	23
Intr	40462	40695	+	23
Intr	40815	41070	+	23
Intr	41176	41255	+	23
Intr	42212	42419	+	23
Intr	42940	43070	+	23
Intr	43177	43410	+	23
Intr	43580	43835	+	23
Intr	46672	46715	+	23
Intr	48334	48532	+	23

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to (I) polynucleotides and methods of use thereof, such as

- IA. Probes, Primers and Substrates;
- IB. Methods of Detection and Isolation;
  - B.1. Hybridization;
  - B.2. Methods of Mapping;
  - B.3. Southern Blotting;
  - B.4. Isolating cDNA from Related Organisms;
  - B.5. Isolating and/or Identifying Orthologous Genes
- IC. Methods of Inhibiting Gene Expression
  - C.1. Antisense
  - C.2. Ribozyme Constructs;
  - C.3. Chimeraplasts;
  - C.4 Co-Suppression;
  - C.5. Transcriptional Silencing
  - C.6. Other Methods to Inhibit Gene Expression
- ID. Methods of Functional Analysis;
- IE. Promoter Sequences and Their Use;
- IF. UTRs and/or Intron Sequences and Their Use; and

IG. Coding Sequences and Their Use.

The invention also relates to (II) polypeptides and proteins and methods of use thereof, such as IIA. Native Polypeptides and Proteins

- A.1 Antibodies
- A.2 In Vitro Applications

IIB. Polypeptide Variants, Fragments and Fusions

- B.1 Variants
- B.2 Fragments
- B.3 Fusions

The invention also includes (III) methods of modulating polypeptide production, such as

IIIA. Suppression

- A.1 Antisense
- A.2 Ribozymes
- A.3 Co-suppression
- A.4 Insertion of Sequences into the Gene to be Modulated
- A.5 Promoter Modulation
- A.6 Expression of Genes containing Dominant-Negative Mutations

IIIB. Enhanced Expression

- B.1 Insertion of an Exogenous Gene
- B.2 Promoter Modulation

The invention further concerns (IV) gene constructs and vector construction, such as

IVA. Coding Sequences

IVB. Promoters

IVC. Signal Peptides

The invention still further relates to

V Transformation Techniques

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Definitions

Allelic variant An "allelic variant" is an alternative form of the same SDF, which resides at the same chromosomal locus in the organism. Allelic variations can occur in any portion of the gene sequence, including regulatory regions. Allelic variants can arise by normal genetic variation in a population. Allelic variants can also be produced by genetic engineering methods. An allelic variant can be one that is found in a naturally occurring plant, including a cultivar or ecotype. An allelic variant may or may not give rise to a phenotypic change, and may or may not be expressed. An allele can result in a detectable change in the phenotype of the trait represented by the locus. A phenotypically silent allele can give rise to a product.

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Alternatively spliced messages Within the context of the current invention, "alternatively spliced messages" refers to mature mRNAs originating from a single gene with variations in the number and/or identity of exons, introns and/or intron-exon junctions.

Chimeric The term "chimeric" is used to describe genes, as defined supra, or contructs wherein at least two of the elements of the gene or construct, such as the promoter and the coding sequence and/or other regulatory sequences and/or filler sequences and/or complements thereof, are heterologous to each other.

Constitutive Promoter: Promoters referred to herein as "constitutive promoters" actively promote transcription under most, but not necessarily all, environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcript initiation region and the 1' or 2' promoter derived from T-DNA of *Agrobacterium tumefaciens,* and other transcription initiation regions from various plant genes, such as the maize ubiquitin-1 promoter, known to those of skill.

Coordinately Expressed: The term "coordinately expressed," as used in the current invention, refers to genes that are expressed at the same or a similar time and/or stage and/or under the same or similar environmental conditions.

Domain: Domains are fingerprints or signatures that can be used to characterize protein families and/or parts of proteins. Such fingerprints or signatures can comprise conserved (1) primary sequence, (2) secondary structure, and/or (3) three-dimensional conformation. Generally, each domain has been associated with either a family of proteins or motifs. Typically, these families and/or motifs have been correlated with specific *in-vitro* and/or *in-vivo* activities. A domain can be any length, including the entirety of the sequence of a protein. Detailed descriptions of the domains, associated families and motifs, and correlated activities of the polypeptides of the instant invention are described below. Usually, the polypeptides with designated domain(s) can exhibit at least one activity that is exhibited by any polypeptide that comprises the same domain(s).

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Endogenous The term "endogenous," within the context of the current invention refers to any polynucleotide, polypeptide or protein sequence which is a natural part of a cell or organisms regenerated from said cell.

Exogenous "Exogenous," as referred to within, is any polynucleotide, polypeptide or protein sequence, whether chimeric or not, that is initially or subsequently introduced into the genome of an individual host cell or the organism regenerated from said host cell by any means other than by a sexual cross. Examples of means by which this can be accomplished are described below, and include *Agrobacterium*-mediated transformation (of dicots - *e.g.* Salomon et al. *EMBO J.* 3:141 (1984); Herrera-Estrella et al. *EMBO J.* 2:987 (1983); of monocots, representative papers are those by Escudero et al., *Plant J.* 10:355 (1996), Ishida et al., *Nature Biotechnology* 14:745 (1996), May et al., *Bio/Technology* 13:486 (1995)), biolistic methods (Armaleo et al., *Current Genetics* 17:97 1990)), electroporation, *in planta* techniques, and the like. Such a plant containing the exogenous nucleic acid is referred to here as a T_0 for the primary transgenic plant and T_1 for the first generation. The term "exogenous" as used herein is also intended to encompass inserting a naturally found element into a non-naturally found location.

Filler sequence: As used herein, "filler sequence" refers to any nucleotide sequence that is inserted into DNA construct to evoke a particular spacing between particular components such as a promoter and a coding region and may provide an additional attribute such as a restriction enzyme site.

Gene: The term “gene,” as used in the context of the current invention, encompasses all regulatory and coding sequence contiguously associated with a single hereditary unit with a genetic function (see SCHEMATIC 1). Genes can include non-coding sequences that modulate the genetic function that include, but are not limited to, those that specify polyadenylation, transcriptional regulation, DNA conformation, chromatin conformation, extent and position of base methylation and binding sites of proteins that control all of these. Genes comprised of “exons” (coding sequences), which may be interrupted by “introns” (non-coding sequences), encode proteins. A gene’s genetic function may require only RNA expression or protein production, or may only require binding of proteins and/or nucleic acids without associated expression. In certain cases, genes adjacent to one another may share sequence in such a way that one gene will overlap the other. A gene can be found within the genome of an organism, artificial chromosome, plasmid, vector, etc., or as a separate isolated entity.

Gene Family: “Gene family” is used in the current invention to describe a group of functionally related genes, each of which encodes a separate protein.

Heterologous sequences: “Heterologous sequences” are those that are not operatively linked or are not contiguous to each other in nature. For example, a promoter from corn is considered heterologous to an *Arabidopsis* coding region sequence. Also, a promoter from a gene encoding a growth factor from corn is considered heterologous to a sequence encoding the corn receptor for the growth factor. Regulatory element sequences, such as UTRs or 3’ end termination sequences that do not originate in nature from the same gene as the coding sequence originates from, are considered heterologous to said coding sequence. Elements operatively linked in nature and contiguous to each other are not heterologous to each other. On the other hand, these same elements remain operatively linked but become heterologous if other filler sequence is placed between them. Thus, the promoter and coding sequences of a corn gene expressing an amino acid transporter are not heterologous to each other, but the promoter and coding sequence of a corn gene operatively linked in a novel manner are heterologous.

Homologous gene In the current invention, “homologous gene” refers to a gene that shares sequence similarity with the gene of interest. This similarity may be in only a fragment of the sequence and often represents a functional domain such as, examples including without limitation a DNA binding domain, a domain with tyrosine kinase activity, or the like. The functional activities of homologous genes are not necessarily the same.

Inducible Promoter An “inducible promoter” in the context of the current invention refers to a promoter which is regulated under certain conditions, such as light, chemical concentration, protein concentration, conditions in an organism, cell, or organelle, etc. A typical example of an inducible promoter, which can be utilized with the polynucleotides of the present invention, is PARSK1, the promoter from the *Arabidopsis* gene encoding a serine-threonine kinase enzyme, and which promoter is induced by dehydration, abscissic acid and sodium chloride (Wang and Goodman, *Plant J.* 8:37 (1995)) Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light.

Intergenic region “Intergenic region,” as used in the current invention, refers to nucleotide sequence occurring in the genome that separates adjacent genes.

Mutant gene In the current invention, “mutant” refers to a heritable change in DNA sequence at a specific location. Mutants of the current invention may or may not have an associated identifiable function when the mutant gene is transcribed.

Orthologous Gene In the current invention “orthologous gene” refers to a second gene that encodes a gene product that performs a similar function as the product of a first gene. The orthologous gene may also have a degree of sequence similarity to the first gene. The orthologous gene may encode a polypeptide that exhibits a degree of sequence similarity to a polypeptide corresponding to a first gene. The sequence similarity can be found within a functional domain or along the entire length of the coding sequence of the genes and/or their corresponding polypeptides.

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Percentage of sequence identity "Percentage of sequence identity," as used herein, is determined by comparing two optimally aligned sequences over a comparison window, where the fragment of the polynucleotide or amino acid sequence in the comparison window may comprise additions or deletions (e.g., gaps or overhangs) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Add. APL. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (USA)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, PASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection. Given that two sequences have been identified for comparison, GAP and BESTFIT are preferably employed to determine their optimal alignment. Typically, the default values of 5.00 for gap weight and 0.30 for gap weight length are used. The term "substantial sequence identity" between polynucleotide or polypeptide sequences refers to polynucleotide or polypeptide comprising a sequence that has at least 80% sequence identity, preferably at least 85%, more preferably at least 90% and most preferably at least 95%, even more preferably, at least 96%, 97%, 98% or 99% sequence identity compared to a reference sequence using the programs.

Plant Promoter A "plant promoter" is a promoter capable of initiating transcription in plant cells and can drive or facilitate transcription of a fragment of the SDF of the instant invention or a coding sequence of the SDF of the instant invention. Such promoters need not be of plant origin. For example, promoters derived from plant viruses, such as the CaMV35S promoter or from *Agrobacterium tumefaciens* such as the T-DNA promoters, can be plant promoters. A typical example of a plant promoter of plant origin is the maize ubiquitin-1 (ubi-1)promoter known to those of skill.

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Promoter: The term "promoter," as used herein, refers to a region of sequence determinants located upstream from the start of transcription of a gene and which are involved in recognition and binding of RNA polymerase and other proteins to initiate and modulate transcription. A basal promoter is the minimal sequence necessary for assembly of a transcription complex required for transcription initiation. Basal promoters frequently include a “TATA box” element usually located between 15 and 35 nucleotides upstream from the site of initiation of transcription. Basal promoters also sometimes include a “CCAAT box” element (typically a sequence CCAAT) and/or a GGGCG sequence, usually located between 40 and 200 nucleotides, preferably 60 to 120 nucleotides, upstream from the start site of transcription.

Public sequence: The term “public sequence ,” as used in the context of the instant application, refers to any sequence that has been deposited in a publicly accessible database. This term encompasses both amino acid and nucleotide sequences. Such sequences are publicly accessible, for example, on the BLAST databases on the NCBI FTP web site (accessible at ncbi.nlm.gov/blast). The database at the NCBI GTP site utilizes “gi” numbers assigned by NCBI as a unique identifier for each sequence in the databases, thereby providing a non-redundant database for sequence from various databases, including GenBank, EMBL, DBBJ, (DNA Database of Japan) and PDB (Brookhaven Protein Data Bank).

Regulatory Sequence The term “regulatory sequence,” as used in the current invention, refers to any nucleotide sequence that influences transcription or translation initiation and rate, and stability and/or mobility of the transcript or polypeptide product. Regulatory sequences include, but are not limited to, promoters, promoter control elements, protein binding sequences, 5’ and 3’ UTRs, transcriptional start site, termination sequence, polyadenylation sequence, introns, certain sequences within a coding sequence, etc.

Related Sequences: “Related sequences” refer to either a polypeptide or a nucleotide sequence that exhibits some degree of sequence similarity with a sequence described in Table 1.

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Scaffold Attachment Region (SAR) As used herein, “scaffold attachment region” is a DNA sequence that anchors chromatin to the nuclear matrix or scaffold to generate loop domains that can have either a transcriptionally active or inactive structure (Spiker and Thompson (1996) Plant Physiol. 110: 15-21).

Sequence-determined DNA fragments (SDFs) “Sequence-determined DNA fragments” as used in the current invention are isolated sequences of genes, fragments of genes, intergenic regions or contiguous DNA from plant genomic DNA or cDNA or RNA the sequence of which has been determined.

Signal Peptide A “signal peptide” as used in the current invention is an amino acid sequence that targets the protein for secretion, for transport to an intracellular compartment or organelle or for incorporation into a membrane. Signal peptides are indicated in the tables and a more detailed description located below.

Specific Promoter In the context of the current invention, “specific promoters” refers to a subset of inducible promoters that have a high preference for being induced in a specific tissue or cell and/or at a specific time during development of an organism. By “high preference” is meant at least 3-fold, preferably 5-fold, more preferably at least 10-fold still more preferably at least 20-fold, 50-fold or 100-fold increase in transcription in the desired tissue over the transcription in any other tissue. Typical examples of temporal and/or tissue specific promoters of plant origin that can be used with the polynucleotides of the present invention, are: PTA29, a promoter which is capable of driving gene transcription specifically in tapetum and only during anther development (Koltonow et al., *Plant Cell* 2:1201 (1990); RCc2 and RCc3, promoters that direct root-specific gene transcription in rice (Xu et al., *Plant Mol. Biol.* 27:237 (1995); TobRB27, a root-specific promoter from tobacco (Yamamoto et al., *Plant Cell* 3:371 (1991)). Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues or organs, such as root, ovule, fruit, seeds, or flowers. Other suitable promoters include those from genes encoding storage proteins or the lipid body membrane protein, oleosin. A few root-specific promoters are noted above.

Stringency "Stringency" as used herein is a function of probe length, probe composition (G + C content), and salt concentration, organic solvent concentration, and temperature of hybridization or wash conditions. Stringency is typically compared by the parameter T_m, which is the temperature at which 50% of the complementary molecules in the hybridization are hybridized, in terms of a temperature differential from T_m. High stringency conditions are those providing a condition of T_m - 5°C to T_m - 10°C. Medium or moderate stringency conditions are those providing T_m - 20°C to T_m - 29°C. Low stringency conditions are those providing a condition of T_m - 40°C to T_m - 48°C. The relationship of hybridization conditions to T_m (in °C) is expressed in the mathematical equation

$$T_m = 81.5 -16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N) \quad (1)$$

where N is the length of the probe. This equation works well for probes 14 to 70 nucleotides in length that are identical to the target sequence. The equation below for T_m of DNA-DNA hybrids is useful for probes in the range of 50 to greater than 500 nucleotides, and for conditions that include an organic solvent (formamide).

$$T_m = 81.5+16.6 \log \{[Na^+]/(1+0.7[Na^+])\}+ 0.41(\%G+C)-500/L\ 0.63(\%formamide) \quad (2)$$

where L is the length of the probe in the hybrid. (P. Tijessen, "Hybridization with Nucleic Acid Probes" in Laboratory Techniques in Biochemistry and Molecular Biology, P.C. vand der Vliet, ed., c. 1993 by Elsevier, Amsterdam.) The T_m of equation (2) is affected by the nature of the hybrid; for DNA-RNA hybrids T_m is 10-15°C higher than calculated, for RNA-RNA hybrids T_m is 20-25°C higher. Because the T_m decreases about 1 °C for each 1% decrease in homology when a long probe is used (Bonner et al., *J. Mol. Biol.* 81:123 (1973)), stringency conditions can be adjusted to favor detection of identical genes or related family members.

Equation (2) is derived assuming equilibrium and therefore, hybridizations according to the present invention are most preferably performed under conditions of probe excess and for sufficient time to achieve equilibrium. The time required to reach equilibrium can be shortened by inclusion of a hybridization accelerator such as dextran sulfate or another high volume polymer in the hybridization buffer.

Stringency can be controlled during the hybridization reaction or after hybridization has occurred by altering the salt and temperature conditions of the wash solutions used. The formulas shown above are equally valid when used to compute the stringency of a wash solution. Preferred wash solution stringencies lie within the ranges stated above; high stringency is 5-8°C below T_m, medium or moderate stringency is 26-29°C below T_m and low stringency is 45-48°C below T_m.

Substantially free of	A composition containing A is "substantially free of " B when at least 85% by weight of the total A+B in the composition is A. Preferably, A comprises at least about 90% by weight of the total of A+B in the composition, more preferably at least about 95% or even 99% by weight. For example, a plant gene or DNA sequence can be considered substantially free of other plant genes or DNA sequences.
Translational start site	In the context of the current invention, a "translational start site" is usually an ATG in the cDNA transcript, more usually the first ATG. A single cDNA, however, may have multiple translational start sites.
Transcription start site	"Transcription start site" is used in the current invention to describe the point at which transcription is initiated. This point is typically located about 25 nucleotides downstream from a TFIID binding site, such as a TATA box. Transcription can initiate at one or more sites within the gene, and a single gene may have multiple transcriptional start sites, some of which may be specific for transcription in a particular cell-type or tissue.
Untranslated region (UTR)	A "UTR" is any contiguous series of nucleotide bases that is transcribed, but is not translated. These untranslated regions may be associated with particular functions such as increasing mRNA message stability. Examples of UTRs include, but are not limited to polyadenylation signals, terminations sequences, sequences located between the transcriptional start site and the first exon (5' UTR) and sequences located between the last exon and the end of the mRNA (3' UTR).
Variant:	The term "variant" is used herein to denote a polypeptide or protein or polynucleotide molecule that differs from others of its kind in some way. For example, polypeptide and protein variants can consist of changes in amino acid sequence and/or charge and/or post-translational modifications (such as glycosylation, etc).

DETAILED DESCRIPTION OF THE INVENTION

I. Polynucleotides

Exemplified SDFs of the invention represent fragments of the genome of corn, wheat, rice, soybean or *Arabidopsis* and/or represent mRNA expressed from that genome. The isolated nucleic acid of the invention also encompasses corresponding fragments of the genome and/or cDNA complement of other organisms as described in detail below.

Polynucleotides of the invention can be isolated from polynucleotide libraries using primers comprising sequence similar to those described by Table 1. See, for example, the methods described in Sambrook et al., supra.

Alternatively, the polynucleotides of the invention can be produced by chemical synthesis. Such synthesis methods are described below.

It is contemplated that the nucleotide sequences presented herein may contain some small percentage of errors. These errors may arise in the normal course of determination of nucleotide sequences. Sequence errors can be corrected by obtaining seeds deposited under the accession numbers cited herein, propagating them, isolating genomic DNA or appropriate mRNA from the resulting plants or seeds thereof, amplifying the relevant fragment of the genomic DNA or mRNA using primers having a sequence that flanks the erroneous sequence, and sequencing the amplification product.

I.A. Probes, Primers and Substrates

SDFs of the invention can be applied to substrates for use in array applications such as, but not limited to, assays of global gene expression, for example under varying conditions of development, growth conditions. The arrays can also be used in diagnostic or forensic methods (WO95/35505, US 5,445,943 and US 5,410,270).

Probes and primers of the instant invention will hybridize to a polynucleotide comprising a sequence in Table 1. Though many different nucleotide sequences can encode an amino acid sequence, the sequences of Table 1 are generally preferred for encoding polypeptides of the invention. However, the sequence of the probes and/or primers of the instant invention need not be identical to those in Table 1 or the complements thereof. For example, some variation in probe or primer sequence and/or length can allow additional family members to be detected, as well as orthologous genes and more taxonomically distant related sequences. Similarly, probes and/or primers of the invention can include additional nucleotides that serve as a label for detecting the formed duplex or for subsequent cloning purposes.

Probe length will vary depending on the application. For use as primers, probes are 12-40 nucleotides, preferably 18-30 nucleotides long. For use in mapping, probes are preferably 50 to 500 nucleotides, preferably 100-250 nucleotides long. For Southern hybridizations, probes as long as several kilobases can be used as explained below.

The probes and/or primers can be produced by synthetic procedures such as the triester method of Matteucci et al. *J. Am. Chem. Soc.* 103:3185(1981); or according to Urdea et al. *Proc. Natl. Acad.* 80:7461 (1981) or using commercially available automated oligonucleotide synthesizers.

I.B. Methods of Detection and Isolation

The polynucleotides of the invention can be utilized in a number of methods known to those skilled in the art as probes and/or primers to isolate and detect polynucleotides, including, without limitation: Southerns, Northerns, Branched DNA hybridization assays, polymerase chain reaction, and microarray assays, and variations thereof. Specific methods given by way of examples, and discussed below include:

Hybridization
Methods of Mapping
Southern Blotting
Isolating cDNA from Related Organisms
Isolating and/or Identifying Orthologous Genes.

Also, the nucleic acid molecules of the invention can used in other methods, such as high density oligonucleotide hybridizing assays, described, for example, in U.S. Pat. Nos. 6,004,753; 5,945,306; 5,945,287; 5,945,308; 5,919,686; 5,919,661; 5,919,627; 5,874,248; 5,871,973; 5,871,971; and 5,871,930; and PCT Pub. Nos. WO 9946380; WO 9933981; WO 9933870; WO 9931252; WO 9915658; WO 9906572; WO 9858052; WO 9958672; and WO 9810858.

B.1. Hybridization

The isolated SDFs of Table 1 of the present invention can be used as probes and/or primers for detection and/or isolation of related polynucleotide sequences through hybridization. Hybridization of one nucleic acid to another constitutes a physical property that defines the subject SDF of the invention and the identified related sequences. Also, such hybridization imposes structural limitations on the pair. A good general discussion of the factors for determining hybridization conditions is provided by Sambrook et al. ("Molecular Cloning, a Laboratory Manual, 2nd ed., c. 1989 by Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; *see esp.,* chapters 11 and 12). Additional considerations and details of the physical chemistry of hybridization are provided by G.H. Keller and M.M. Manak "DNA Probes", 2nd Ed. pp. 1-25, c. 1993 by Stockton Press, New York, NY.

Depending on the stringency of the conditions under which these probes and/or primers are used, polynucleotides exhibiting a wide range of similarity to those in Table 1 can be detected or isolated. When the practitioner wishes to examine the result of membrane hybridizations under a variety of stringencies, an efficient way to do so is to perform the hybridization under a low stringency condition, then to wash the hybridization membrane under increasingly stringent conditions.

When using SDFs to identify orthologous genes in other species, the practitioner will preferably adjust the amount of target DNA of each species so that, as nearly as is practical, the same number of genome equivalents are present for each species examined. This prevents faint signals from species having large genomes, and thus small numbers of genome equivalents per mass of DNA, from erroneously being interpreted as absence of the corresponding gene in the genome.

The probes and/or primers of the instant invention can also be used to detect or isolate nucleotides that are "identical" to the probes or primers. Two nucleic acid sequences or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below.

Isolated polynucleotides within the scope of the invention also include allelic variants of the specific sequences presented in Table 1. The probes and/or primers of the invention can also be used to detect and/or isolate polynucleotides exhibiting at least 80% sequence identity with the sequences of Table 1 or fragments thereof.

With respect to nucleotide sequences, degeneracy of the genetic code provides the possibility to substitute at least one base of the base sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, the DNA of the present invention may also have any base sequence that has been changed from a sequence in Table 1 by substitution in accordance with degeneracy of genetic code. References describing codon usage include: Carels *et al., J. Mol. Evol.* 46: 45 (1998) and Fennoy *et al., Nucl. Acids Res.* 21(23): 5294 (1993).

B.2. Mapping

The isolated SDF DNA of the invention can be used to create various types of genetic and physical maps of the genome of corn, Arabidopsis, soybean, rice, wheat, or other plants. Some SDFs may be absolutely associated with particular phenotypic traits, allowing construction of gross genetic maps. While not all SDFs will immediately be associated with a phenotype, all SDFs can be used as probes for identifying polymorphisms associated with phenotypes of interest. Briefly, one method of mapping involves total DNA isolation from individuals. It is subsequently cleaved with one or more restriction enzymes, separated according to mass, transferred to a solid support, hybridized with SDF DNA and the pattern of fragments compared. Polymorphisms associated with a particular SDF are visualized as differences in the size of fragments produced between individual DNA samples after digestion with a particular restriction enzyme and hybridization with the SDF. After identification of polymorphic SDF sequences, linkage studies can be conducted. By using the individuals showing polymorphisms as parents in crossing programs, F2 progeny recombinants or recombinant inbreds, for example, are then analyzed. The order of DNA polymorphisms along the chromosomes can be determined based on the frequency with which they are inherited together versus independently. The closer two polymorphisms are together in a chromosome the higher the probability that they are inherited together. Integration of the relative positions of all the polymorphisms and associated marker SDFs can produce a genetic map of the species, where the distances between markers reflect the recombination frequencies in that chromosome segment.

The use of recombinant inbred lines for such genetic mapping is described for *Arabidopsis* by Alonso-Blanco et al. (*Methods in Molecular Biology*, vol.82, "*Arabidopsis Protocols*", pp. 137-146, J.M. Martinez-Zapater and J. Salinas, eds., c. 1998 by Humana Press, Totowa, NJ) and for corn by Burr ("Mapping Genes with Recombinant Inbreds", pp.

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249-254. *In* Freeling, M. and V. Walbot (Ed.), *The Maize Handbook*, c. 1994 by Springer-Verlag New York, Inc.: New York, NY, USA; Berlin Germany; Burr et al. *Genetics* (1998) 118: 519; Gardiner, J. et al., (1993) *Genetics* 134: 917). This procedure, however, is not limited to plants and can be used for other organisms (such as yeast) or for individual cells.

The SDFs of the present invention can also be used for simple sequence repeat (SSR) mapping. Rice SSR mapping is described by Morgante et al. (*The Plant Journal* (1993) 3: 165), Panaud et al. (*Genome* (1995) 38: 1170); Senior et al. (*Crop Science* (1996) 36: 1676), Taramino et al. (*Genome* (1996) 39: 277) and Ahn et al. (*Molecular and General Genetics* (1993) 241: 483-90). SSR mapping can be achieved using various methods. In one instance, polymorphisms are identified when sequence specific probes contained within an SDF flanking an SSR are made and used in polymerase chain reaction (PCR) assays with template DNA from two or more individuals of interest. Here, a change in the number of tandem repeats between the SSR-flanking sequences produces differently sized fragments (U.S. Patent 5,766,847). Alternatively, polymorphisms can be identified by using the PCR fragment produced from the SSR-flanking sequence specific primer reaction as a probe against Southern blots representing different individuals (U.H. Refseth et al., (1997) *Electrophoresis* 18: 1519).

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Genetic and physical maps of crop species have many uses. For example, these maps can be used to devise positional cloning strategies for isolating novel genes from the mapped crop species. In addition, because the genomes of closely related species are largely syntenic (that is, they display the same ordering of genes within the genome), these maps can be used to isolate novel alleles from relatives of crop species by positional cloning strategies.

The various types of maps discussed above can be used with the SDFs of the invention to identify Quantitative Trait Loci (QTLs). Many important crop traits, such as the solids content of tomatoes, are quantitative traits and result from the combined interactions of several genes. These genes reside at different loci in the genome, oftentimes on different chromosomes, and generally exhibit multiple alleles at each locus. The SDFs of the invention can be used to identify QTLs and isolate specific alleles as described by de Vicente and Tanksley (*Genetics* 134:585 (1993)). In addition to isolating QTL alleles in present crop species, the SDFs of the invention can also be used to isolate alleles from the corresponding QTL of wild relatives. Transgenic plants having various combinations of QTL alleles can then be created and the effects of the combinations measured. Once a desired allele combination has been identified, crop improvement can be accomplished either through biotechnological means or by directed conventional breeding programs (for review *see* Tanksley and McCouch, *Science* 277:1063 (1997)).

In another embodiment, the SDFs can be used to help create physical maps of the genome of corn, *Arabidopsis* and related species. Where SDFs have been ordered on a genetic map, as described above, they can be used as probes to discover which clones in large libraries of plant DNA fragments in YACs, BACs, etc. contain the same SDF or similar sequences, thereby facilitating the assignment of the large DNA fragments to chromosomal positions. Subsequently, the large BACs, YACs, etc. can be ordered unambiguously by more detailed studies of their sequence composition (e.g. Marra et al. (1997) Genomic Research 7:1072-1084) and by using their end or other sequences to find the identical sequences in other cloned DNA fragments. The overlapping of DNA sequences in this way allows large contigs of plant sequences to be built that, when sufficiently extended, provide a complete physical map of a chromosome. Sometimes the SDFs themselves will provide the means of joining cloned sequences into a contig.

The patent publication WO95/35505 and U.S. Patents 5,445,943 and 5,410,270 describe scanning multiple alleles of a plurality of loci using hybridization to arrays of oligonucleotides. These techniques are useful for each of the types of mapping discussed above.

Following the procedures described above and using a plurality of the SDFs of the present invention, any individual can be genotyped. These individual genotypes can be used for the identification of particular cultivars, varieties, lines, ecotypes and genetically modified plants or can serve as tools for subsequent genetic studies involving multiple phenotypic traits.

B.3 Southern Blot Hybridization

The sequences from Table 1 can be used as probes for various hybridization techniques. These techniques are useful for detecting target polynucleotides in a sample or for determining whether transgenic plants, seeds or host cells harbor a gene or sequence of interest and thus might be expected to exhibit a particular trait or phenotype.

In addition, the SDFs from the invention can be used to isolate additional members of gene families from the same or different species and/or orthologous genes from the same or different species. This is accomplished by hybridizing an SDF to, for example, a Southern blot containing the appropriate genomic DNA or cDNA. Given the resulting hybridization

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data, one of ordinary skill in the art could distinguish and isolate the correct DNA fragments by size, restriction sites, sequence and stated hybridization conditions from a gel or from a library.

Identification and isolation of orthologous genes from closely related species and alleles within a species is particularly desirable because of their potential for crop improvement. Many important crop traits, such as the solid content of tomatoes, result from the combined interactions of the products of several genes residing at different loci in the genome. Generally, alleles at each of these loci can make quantitative differences to the trait. By identifying and isolating numerous alleles for each locus from within or different species, transgenic plants with various combinations of alleles can be created and the effects of the combinations measured. Once a more favorable allele combination has been identified, crop improvement can be accomplished either through biotechnological means or by directed conventional breeding programs (Tanksley et al. *Science* 277:1063(1997)).

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The results from hybridizations of the SDFs of the invention to, for example, Southern blots containing DNA from another species can also be used to generate restriction fragment maps for the corresponding genomic regions. These maps provide additional information about the relative positions of restriction sites within fragments, further distinguishing mapped DNA from the remainder of the genome.

Physical maps can be made by digesting genomic DNA with different combinations of restriction enzymes.

Probes for Southern blotting to distinguish individual restriction fragments can range in size from 15 to 20 nucleotides to several thousand nucleotides. More preferably, the probe is 100 to 1,000 nucleotides long for identifying members of a gene family when it is found that repetitive sequences would complicate the hybridization. For identifying an entire corresponding gene in another species, the probe is more preferably the length of the gene, typically 2,000 to 10,000 nucleotides, but probes 50-1,000 nucleotides long might be used. Some genes, however, might require probes up to 1,500 nucleotides long or overlapping probes constituting the full-length sequence to span their lengths.

Also, while it is preferred that the probe be homogeneous with respect to its sequence, it is not necessary. For example, as described below, a probe representing members of a gene family having diverse sequences can be generated using PCR to amplify genomic DNA or

RNA templates using primers derived from SDFs that include sequences that define the gene family.

For identifying corresponding genes in another species, the next most preferable probe is a cDNA spanning the entire coding sequence, which allows all of the mRNA-coding fragment of the gene to be identified. Probes for Southern blotting can easily be generated from SDFs by making primers having the sequence at the ends of the SDF and using corn or *Arabidopsis* genomic DNA as a template. In instances where the SDF includes sequence conserved among species, primers including the conserved sequence can be used for PCR with genomic DNA from a species of interest to obtain a probe. Similarly, if the SDF includes a domain of interest, that fragment of the SDF can be used to make primers and, with appropriate template DNA, used to make a probe to identify genes containing the domain. Alternatively, the PCR products can be resolved, for example by gel electrophoresis, and cloned and/or sequenced. Using Southern hybridization, the variants of the domain among members of a gene family, both within and across species, can be examined.

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B.4.1 Isolating DNA from Related Organisms

The SDFs of the invention can be used to isolate the corresponding DNA from other organisms. Either cDNA or genomic DNA can be isolated. For isolating genomic DNA, a lambda, cosmid, BAC or YAC, or other large insert genomic library from the plant of interest can be constructed using standard molecular biology techniques as described in detail by Sambrook et al. 1989 (Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, New York) and by Ausubel et al. 1992 (Current Protocols in Molecular Biology, Greene Publishing, New York).

To screen a phage library, for example, recombinant lambda clones are plated out on appropriate bacterial medium using an appropriate *E. coli* host strain. The resulting plaques are lifted from the plates using nylon or nitrocellulose filters. The plaque lifts are processed through denaturation, neutralization, and washing treatments following the standard protocols outlined by Ausubel et al. (1992). The plaque lifts are hybridized to either radioactively labeled or non-radioactively labeled SDF DNA at room temperature for about 16 hours, usually in the presence of 50% formamide and 5X SSC (sodium chloride and sodium citrate) buffer and blocking reagents. The plaque lifts are then washed at 42°C with 1% Sodium Dodecyl Sulfate (SDS) and at a particular concentration of SSC. The SSC concentration used is dependent upon the stringency at which hybridization occurred in the initial Southern blot analysis performed. For example, if a fragment hybridized under medium stringency (e.g., Tm - 20°C), then this condition is maintained or preferably adjusted to a less stringent condition (e.g., Tm-30°C) to wash the plaque lifts. Positive clones show detectable hybridization e.g., by exposure to X-ray films or chromogen formation. The positive clones are then subsequently isolated for purification using the same general protocol outlined above. Once the clone is purified, restriction analysis can be conducted to narrow the region corresponding to the gene of interest. The restriction analysis and succeeding subcloning steps can be done using procedures described by, for example Sambrook et al. (1989) cited above.

The procedures outlined for the lambda library are essentially similar to those used for YAC library screening, except that the YAC clones are harbored in bacterial colonies. The YAC clones are plated out at reasonable density on nitrocellulose or nylon filters supported by appropriate bacterial medium in petri plates. Following the growth of the bacterial clones, the filters are processed through the denaturation, neutralization, and washing steps following the procedures of Ausubel et al. 1992. The same hybridization procedures for lambda library screening are followed.

To isolate cDNA, similar procedures using appropriately modified vectors are employed. For instance, the library can be constructed in a lambda vector appropriate for cloning cDNA such as λgt11. Alternatively, the cDNA library can be made in a plasmid vector. cDNA for cloning can be prepared by any of the methods known in the art, but is preferably prepared as described above. Preferably, a cDNA library will include a high proportion of full-length clones.

B. 5. Isolating and/or Identifying Orthologous Genes

Probes and primers of the invention can be used to identify and/or isolate polynucleotides related to those in Table 1. Related polynucleotides are those that are native to other plant organisms and exhibit either similar sequence or encode polypeptides with similar biological activity. One specific example is an orthologous gene. Orthologous genes have the same functional activity. As such, orthologous genes may be distinguished from homologous genes. The percentage of identity is a function of evolutionary separation and, in closely related species, the percentage of identity can be 98 to 100%. The amino acid sequence of a protein encoded by an orthologous gene can be less than 75% identical, but tends to be at least75% or at least 80% identical, more preferably at least 90%, most preferably at least 95% identical to the amino acid sequence of the reference protein.

To find orthologous genes, the probes are hybridized to nucleic acids from a species of interest under low stringency conditions, preferably one where sequences containing as much as 40-45% mismatches will be able to hybridize. This condition is established by T_m - 40°C to Tm - 48°C (*see* below). Blots are then washed under conditions of increasing stringency. It is preferable that the wash stringency be such that sequences that are 85 to 100% identical will hybridize. More preferably, sequences 90 to 100% identical will hybridize and most preferably only sequences greater than 95% identical will hybridize. One of ordinary skill in the art will recognize that, due to degeneracy in the genetic code, amino acid sequences that are identical can be encoded by DNA sequences as little as 67% identical or less. Thus, it is preferable, for example, to make an overlapping series of shorter probes, on the order of 24 to 45 nucleotides, and individually hybridize them to the same arrayed library to avoid the problem of degeneracy introducing large numbers of mismatches.

As evolutionary divergence increases, genome sequences also tend to diverge. Thus, one of skill will recognize that searches for orthologous genes between more divergent species will require the use of lower stringency conditions compared to searches between closely related species. Also, degeneracy of the genetic code is more of a problem for searches in the genome of a species more distant evolutionarily from the species that is the source of the SDF probe sequences.

Therefore the method described in Bouckaert et al., U.S. Ser. No. 60/121,700 Atty. Dkt. No. 2750-117P, Client Dkt. No. 00010.001, filed February 25, 1999, hereby incorporated in its entirety by reference, can be applied to the SDFs of the present invention to isolate related genes from plant species which do not hybridize to the corn *Arabidopsis,* soybean, rice, wheat, and other plant sequences of Table 1.

Identification of the relationship of nucleotide or amino acid sequences among plant species can be done by comparing the nucleotide or amino acid sequences of SDFs of the present application with nucleotide or amino acid sequences of other SDFs such as those present in applications listed in the table below:

Attorney Docket	Client Docket	Filing Date	Application
2750-0301P	80002.001	9/4/1998	60/099,672
2750-0300P	80001.001	9/4/1998	60/099,671
2750-0302P	80003.001	9/11/1998	60/099,933
2750-0304P	80004.001	9/17/1998	60/100,864
2750-0305P	80005.001	9/18/1998	60/101,042

Attorney Docket	Client Docket	Filing Date	Application
2750-0306P	80006.001	9/21/1998	60/101,255
2750-0307P	80007.001	9/24/1998	60/101,682
2750-0308P	80008.001	9/30/1998	60/102,533
2750-0309P	80009.001	9/30/1998	60/102,460
2750-0310P	80010.001	10/5/1998	60/103,116
2750-0311P	80011.001	10/5/1998	60/103,141
2750-0312P	80012.001	10/6/1998	60/103,215
2750-0313P	80013.001	10/8/1998	60/103,554
2750-0314P	80014.001	10/9/1998	60/103,574
2750-0315P	80015.001	10/13/1998	60/103,907
2750-0316P	80016.001	10/14/1998	60/104,268
2750-0317P	80017.001	10/16/1998	60/104,680
2750-0318P	80018.001	10/19/1998	60/104,828
2750-0319P	80019.001	10/20/1998	60/105,008
2750-0320P	80020.001	10/21/1998	60/105,142
2750-0321P	80021.001	10/22/1998	60/105,533
2750-0322P	80022.001	10/26/1998	60/105,571
2750-0323P	80023.001	10/27/1998	60/105,815
2750-0324P	80024.001	10/29/1998	60/106,105
2750-0325P	80025.001	10/30/1998	60/106,218
2750-0326P	80026.001	11/2/1998	60/106,685
2750-0327P	80027.001	11/6/1998	60/107,282
2750-0329P	80029.001	11/9/1998	60/107,719
2750-0328P	80028.001	11/9/1998	60/107,720
2750-0330P	80030.001	11/10/1998	60/107,836
2750-0331P	80031.001	11/12/1998	60/108,190
2750-0332P	80032.001	11/16/1998	60/108,526
2750-0333P	80033.001	11/17/1998	60/108,901
2750-0335P	80035.001	11/19/1998	60/109,127
2750-0334P	80034.001	11/19/1998	60/109,124
2750-0336P	80036.001	11/20/1998	60/109,267
2750-0337P	80037.001	11/23/1998	60/109,594
2750-0338P	80038.001	11/25/1998	60/110,053
2750-0339P	80039.001	11/25/1998	60/110,050
2750-0340P	80040.001	11/27/1998	60/110,158
2750-0341P	80041.001	11/30/1998	60/110,263
2750-0342P	80042.001	12/1/1998	60/110,495
2750-0343P	80043.001	12/2/1998	60/110,626
2750-0344P	80044.001	12/3/1998	60/110,701
2750-0345P	80045.001	12/7/1998	60/111,339
2750-0346P	80046.001	12/9/1998	60/111,589
2750-0347P	80047.001	12/10/1998	60/111,782
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2750-0349P	80049.001	12/14/1998	60/112,096
2750-0350P	80050.001	12/15/1998	60/112,224
2750-0351P	80051.001	12/16/1998	60/112,624
2750-0352P	80052.001	12/17/1998	60/112,862
2750-0353P	80053.001	12/18/1998	60/112,912
2750-0354P	80054.001	12/21/1998	60/113,248

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2750-0355P	80055.001	12/22/1998	60/113,522
2750-0356P	80056.001	12/23/1998	60/113,826
2750-0357P	80057.001	12/28/1998	60/113,998
2750-0358P	80058.001	12/29/1998	60/114,384
2750-0359P	80059.001	12/30/1998	60/114,455
2750-0360P	80060.001	1/4/1999	60/114,740
2750-0361P	80061.001	1/6/1999	60/114,866
2750-0362P	80062.001	1/7/1999	60/115,153
2750-0367P	80067.001	1/7/1999	60/115,154
2750-0366P	80066.001	1/7/1999	60/115,156
2750-0365P	80065.001	1/7/1999	60/115,155
2750-0363P	80063.001	1/7/1999	60/115,152
2750-0364P	80064.001	1/7/1999	60/115,151
2750-0370P	80070.001	1/8/1999	60/115,293
2750-0369P	80069.001	1/8/1999	60/115,365
2750-0368P	80068.001	1/8/1999	60/115,364
2750-0371P	80071.001	1/11/1999	60/115,339
2750-0372P	80072.001	1/12/1999	60/115,518
2750-0373P	80073.001	1/13/1999	60/115,847
2750-0374P	80074.001	1/14/1999	60/115,905
2750-0375P	80075.001	1/15/1999	60/116,383
2750-0376P	80076.001	1/15/1999	60/116,384
2750-0378P	80078.001	1/19/1999	60/116,340
2750-0377P	80077.001	1/19/1999	60/116,329
2750-0380P	80080.001	1/21/1999	60/116,672
2750-0379P	80079.001	1/21/1999	60/116,674
2750-0381P	80081.001	1/22/1999	60/116,960
2750-0382P	80082.001	1/22/1999	60/116,962
2750-0383P	80083.001	1/28/1999	60/117,756
2750-0384P	80084.001	2/3/1999	60/118,672
2750-0385P	80085.001	2/4/1999	60/118,808
2750-0386P	80086.001	2/5/1999	60/118,778
2750-0387P	80087.001	2/8/1999	60/119,029
2750-0388P	80088.001	2/9/1999	60/119,332
2750-0389P	80089.001	2/10/1999	60/119,462
2750-0391P	80091.001	2/12/1999	60/119,922
2750-0392P	80092.001	2/16/1999	60/120,196
2750-0393P	80093.001	2/16/1999	60/120,198
2750-0394P	80094.001	2/18/1999	60/120,583
2750-0395P	80095.001	2/22/1999	60/121,072
2750-0396P	80096.001	2/23/1999	60/121,334
2750-0397P	80097.001	2/24/1999	60/121,470
2750-0390P	80090.001	2/25/1999	60/121,825
2750-0398P	80098.001	2/25/1999	60/121,704
2750-0399P	80099.001	2/26/1999	60/122,107
2750-0400P	80100.001	3/1/1999	60/122,266
2750-0401P	80101.001	3/2/1999	60/122,568
2750-0402P	80102.001	3/3/1999	60/122,611
2750-0403P	80103.001	3/4/1999	60/121,775

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2750-0405P	80105.001	3/5/1999	60/123,180
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2750-0407P	80107.001	3/9/1999	60/123,548
2750-0408P	80108.001	3/10/1999	60/123,715
2750-0409P	80109.001	3/10/1999	60/123,726
2750-0410P	80110.001	3/11/1999	60/124,263
2750-0411P	80111.001	3/12/1999	60/123,941
2750-0412P	80112.001	3/23/1999	60/125,788
2750-0413P	80113.001	3/25/1999	60/126,264
2750-0414P	80114.001	3/29/1999	60/126,785
2750-0415P	80115.001	4/1/1999	60/127,462
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2750-0417P	91001.001	4/8/1999	60/128,714
2750-0418P	80118.001	4/16/1999	60/129,845
2750-0420P	80120.001	4/19/1999	60/130,077
2750-0421P	80121.001	4/21/1999	60/130,449
2750-0303P	80115.002	4/23/1999	60/130,510
2750-0422P	80122.001	4/23/1999	60/130,891
2750-0423P	80123.001	4/28/1999	60/131,449
2750-0424P	80124.001	4/30/1999	60/132,407
2750-0425P	80125.001	4/30/1999	60/132,048
2750-0426P	80126.001	5/4/1999	60/132,484
2750-0427P	80127.001	5/5/1999	60/132,485
2750-0428P	91002.001	5/6/1999	60/132,487
2750-0429P	80129.001	5/6/1999	60/132,486
2750-0430P	80130.001	5/7/1999	60/132,863
2750-0431P	80131.001	5/11/1999	60/134,256
2750-0433P	00025.001	5/14/1999	60/134,221
2750-0432P	91006.001	5/14/1999	60/134,370
2750-0434P	80116.001	5/14/1999	60/134,219
2750-0435P	80117.001	5/14/1999	60/134,218
2750-0436P	91007.001	5/18/1999	60/134,768
2750-0437P	91008.001	5/19/1999	60/134,941
2750-0438P	91009.001	5/20/1999	60/135,124
2750-0439P	91010.001	5/21/1999	60/135,353
2750-0440P	91011.001	5/24/1999	60/135,629
2750-0441P	91012.001	5/25/1999	60/136,021
2750-0442P	91013.001	5/27/1999	60/136,392
2750-0444P	91014.001	5/28/1999	60/136,782
2750-0445P	91015.001	6/1/1999	60/137,222
2750-0446P	91016.001	6/3/1999	60/137,528
2750-0447P	91017.001	6/4/1999	60/137,502
2750-0449P	91018.001	6/7/1999	60/137,724
2750-0450P	91019.001	6/8/1999	60/138,094
2750-0457P	00033.001	6/10/1999	60/138,540
2750-0458P	00033.002	6/10/1999	60/138,847
2750-0463P	00034.001	6/14/1999	60/139,119
2750-0461P	80132.011	6/16/1999	60/139,453

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2750-0462P	80132.012	6/16/1999	60/139,452
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2750-0453P	80132.005	6/18/1999	60/139,462
2750-0466P	00039.001	6/18/1999	60/139,750
2750-0465P	00038.001	6/18/1999	60/139,763
2750-0460P	80132.010	6/18/1999	60/139,455
2750-0451P	80132.003	6/18/1999	60/139,459
2750-0454P	80132.006	6/18/1999	60/139,457
2750-0459P	80132.009	6/18/1999	60/139,463
2750-0448P	80132.002	6/18/1999	60/139,454
2750-0443P	80132.001	6/18/1999	60/139,458
2750-0456P	80132.008	6/18/1999	60/139,456
2750-0455P	80132.007	6/18/1999	60/139,460
2750-0452P	80132.004	6/18/1999	60/139,461
2750-0467P	00042.001	6/21/1999	60/139,817
2750-0468P	00043.001	6/22/1999	60/139,899
2750-0470P	00042.002	6/23/1999	60/140,353
2750-0469P	00044.001	6/23/1999	60/140,354
2750-0471P	00045.001	6/24/1999	60/140,695
2750-0472P	00046.001	6/28/1999	60/140,823
2750-0473P	00048.001	6/29/1999	60/140,991
2750-0474P	00049.001	6/30/1999	60/141,287
2750-0475P	00050.001	7/1/1999	60/141,842
2750-0476P	00051.001	7/1/1999	60/142,154
2750-0477P	00052.001	7/2/1999	60/142,055
2750-0478P	00053.001	7/6/1999	60/142,390
2750-0479P	00054.001	7/8/1999	60/142,803
2750-0480P	00058.001	7/9/1999	60/142,920
2750-0481P	00059.001	7/12/1999	60/142,977
2750-0482P	00060.001	7/13/1999	60/143,542
2750-0489P	00061.001	7/14/1999	60/143,624
2750-0490P	00062.001	7/15/1999	60/144,005
2750-0485P	80134.003	7/16/1999	60/144,086
2750-0486P	80134.004	7/16/1999	60/144,085
2750-0497P	00064.001	7/19/1999	60/144,325
2750-0496P	80134.014	7/19/1999	60/144,334
2750-0495P	80134.013	7/19/1999	60/144,335
2750-0494P	80134.010	7/19/1999	60/144,333
2750-0492P	80134.008	7/19/1999	60/144,331
2750-0488P	80134.006	7/19/1999	60/144,332
2750-0500P	00065.001	7/20/1999	60/144,632
2750-0502P	80135.002	7/20/1999	60/144,884
2750-0499P	80134.012	7/20/1999	60/144,352
2750-0503P	00066.001	7/21/1999	60/144,814
2750-0483P	80134.001	7/21/1999	60/145,088
2750-0484P	80134.002	7/21/1999	60/145,086
2750-0504P	00067.001	7/22/1999	60/145,192
2750-0491P	80134.007	7/22/1999	60/145,085
2750-0493P	80134.009	7/22/1999	60/145,087

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2750-0487P	80134.005	7/22/1999	60/145,089
2750-0498P	80134.011	7/23/1999	60/145,145
2750-0501P	80135.001	7/23/1999	60/145,224
2750-0505P	00069.001	7/23/1999	60/145,218
2750-0506P	00070.001	7/26/1999	60/145,276
2750-0507P	80136.001	7/27/1999	60/145,918
2750-0508P	80136.002	7/27/1999	60/145,919
2750-0509P	00071.001	7/27/1999	60/145,913
2750-0510P	00072.001	7/28/1999	60/145,951
2750-0513P	00073.001	8/2/1999	60/146,386
2750-0512P	80137.002	8/2/1999	60/146,389
2750-0511P	80137.001	8/2/1999	60/146,388
2750-0514P	00074.001	8/3/1999	60/147,038
2750-0515P	00076.001	8/4/1999	60/147,204
2750-0517P	80138.002	8/4/1999	60/147,302
2750-0519P	80136.003	8/5/1999	60/147,192
2750-0518P	00077.001	8/5/1999	60/147,260
2750-0520P	00079.001	8/6/1999	60/147,416
2750-0516P	80138.001	8/6/1999	60/147,303
2750-0523P	80139.002	8/9/1999	60/147,935
2750-0521P	00080.001	8/9/1999	60/147,493
2750-0522P	80139.001	8/10/1999	60/148,171
2750-0524P	00081.001	8/11/1999	60/148,319
2750-0530P	00082.001	8/12/1999	60/148,341
2750-0525P	80141.001	8/12/1999	60/148,347
2750-0526P	80141.002	8/12/1999	60/148,342
2750-0527P	80141.003	8/12/1999	60/148,340
2750-0528P	80141.004	8/12/1999	60/148,337
2750-0532P	80142.002	8/13/1999	60/148,684
2750-0529P	00083.001	8/13/1999	60/148,565
2750-0531P	80142.001	8/16/1999	60/149,368
2750-0533P	80001.002	8/17/1999	60/149,927
2750-0534P	80001.003	8/17/1999	60/149,928
2750-0535P	80001.004	8/17/1999	60/149,926
2750-0536P	80001.005	8/17/1999	60/149,925
2750-0537P	00084.001	8/17/1999	60/149,175
2750-0538P	00085.001	8/18/1999	60/149,426
2750-0542P	00087.001	8/20/1999	60/149,723
2750-0541P	80143.002	8/20/1999	60/149,929
2750-0539P	00086.001	8/20/1999	60/149,722
2750-0543P	00088.001	8/23/1999	60/149,902
2750-0540P	80143.001	8/23/1999	60/149,930
2750-0544P	00089.001	8/25/1999	60/150,566
2750-0547P	00090.001	8/26/1999	60/150,884
2750-0546P	80144.002	8/27/1999	60/151,066
2750-0548P	00091.001	8/27/1999	60/151,080
2750-0545P	80144.001	8/27/1999	60/151,065
2750-0549P	00092.001	8/30/1999	60/151,303
2750-0552P	00093.001	8/31/1999	60/151,438

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2750-0550P	80001.006	9/3/1999	09/391,631
2750-0551F(PC)	80001.100	9/3/1999	99/204,38
2750-0554P	00095.001	9/7/1999	60/152,363
2750-0555P	00096.001	9/10/1999	60/153,070
2750-0556P	00098.001	9/13/1999	60/153,758
2750-0557P	00099.001	9/15/1999	60/154,018
2750-0558P	00101.001	9/16/1999	60/154,039
2750-0559P	00102.001	9/20/1999	60/154,779
2750-0560P	00103.001	9/22/1999	60/155,139
2750-0561P	00104.001	9/23/1999	60/155,486
2750-0562P	00105.001	9/24/1999	60/155,659
2750-0563P	00106.001	9/28/1999	60/156,458
2750-0564P	00107.001	9/29/1999	60/156,596
2750-0570P	00108.001	10/4/1999	60/157,117
2750-0571P	00109.001	10/5/1999	60/157,753
2750-0565P	80010.002	10/5/1999	09/413,198
2750-0566P	80010.003	10/5/1999	09/412,922
2750-0567F(PC)	80010.100	10/5/1999	99/228,55
2750-0568F(PC)	80010.101	10/5/1999	99/228,54
2750-0569F(PC)	80010.102	10/5/1999	99/228,53
2750-0572P	00110.001	10/6/1999	60/157,865
2750-0575P	00111.001	10/7/1999	60/158,029
2750-0576P	00112.001	10/8/1999	60/158,232
2750-0577P	00113.001	10/12/1999	60/158,369
2750-0574P	80145.002	10/13/1999	60/159,295
2750-0579P	80146.002	10/13/1999	60/159,293
2750-0583P	80148.002	10/13/1999	60/159,294
2750-0573P	80145.001	10/14/1999	60/159,330
2750-0580P	80147.001	10/14/1999	60/159,638
2750-0581P	80147.002	10/14/1999	60/159,637
2750-0582P	80148.001	10/14/1999	60/159,329
2750-0578P	80146.001	10/14/1999	60/159,331
2750-0584P	00116.001	10/18/1999	60/159,584
2750-0586P	80149.001	10/21/1999	60/160,814
2750-0587P	80149.002	10/21/1999	60/160,770
2750-0588P	00119.001	10/21/1999	60/160,741
2750-0589P	80150.001	10/21/1999	60/160,768
2750-0590P	80150.002	10/21/1999	60/160,767
2750-0585P	00118.001	10/21/1999	60/160,815
2750-0593P	80151.002	10/22/1999	60/160,981
2750-0591P	00120.001	10/22/1999	60/160,980
2750-0592P	80151.001	10/22/1999	60/160,989
2750-0596P	80152.002	10/25/1999	60/161,404
2750-0595P	80152.001	10/25/1999	60/161,406
2750-0594P	00121.001	10/25/1999	60/161,405
2750-0597P	00122.001	10/26/1999	60/161,361
2750-0598P	80153.001	10/26/1999	60/161,360
2750-0599P	80153.002	10/26/1999	60/161,359

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2750-0603P	80154.002	10/28/1999	60/161,993
2750-0604P	00124.001	10/29/1999	60/162,143
2750-0605P	80155.001	10/29/1999	60/162,142
2750-0606P	80155.002	10/29/1999	60/162,228
2750-0607P	00125.001	11/1/1999	60/162,894
2750-0608P	80156.001	11/1/1999	60/162,891
2750-0609P	80156.002	11/1/1999	60/162,895
2750-0610P	00126.001	11/2/1999	60/163,093
2750-0611P	80157.001	11/2/1999	60/163,092
2750-0612P	80157.002	11/2/1999	60/163,091
2750-0614P	80158.001	11/3/1999	60/163,248
2750-0615P	80158.002	11/3/1999	60/163,281
2750-0613P	00127.001	11/3/1999	60/163,249
2750-0618P	80159.002	11/4/1999	60/163,380
2750-0617P	80159.001	11/4/1999	60/163,381
2750-0616P	00128.001	11/4/1999	60/163,379
2750-0621P	80160.002	11/8/1999	60/164,150
2750-0620P	80160.001	11/8/1999	60/164,151
2750-0619P	00129.001	11/8/1999	60/164,146
2750-0623P	80161.002	11/9/1999	60/164,260
2750-0625P	80162.002	11/9/1999	60/164,259
2750-0626P	80163.001	11/10/1999	60/164,321
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All applications listed in the table above are expressly incorporated herein by reference in their entirety and for all purposes.

The SDFs of the invention can also be used as probes to search for genes that are related to the SDF within a species. Such related genes are typically considered to be members of a gene family. In such a case, the sequence similarity will often be concentrated into one or a few fragments of the sequence. The fragments of similar sequence that define the gene family typically encode a fragment of a protein or RNA that has an enzymatic or structural function. The percentage of identity in the amino acid sequence of the domain that defines the gene family is preferably at least 70%, more preferably 80 to 95%, most preferably 85 to 99%. To search for members of a gene family within a species, a low stringency hybridization is usually performed, but this will depend upon the size, distribution and degree of sequence divergence of domains that define the gene family. SDFs encompassing regulatory regions can be used to identify coordinately expressed genes by using the regulatory region sequence of the SDF as a probe.

In the instances where the SDFs are identified as being expressed from genes that confer a particular phenotype, then the SDFs can also be used as probes to assay plants of different species for those phenotypes.

I.C. Methods to Inhibit Gene Expression

The nucleic acid molecules of the present invention can be used to inhibit gene transcription and/or translation. Example of such methods include, without limitation:

Antisense Constructs;
Ribozyme Constructs;
Chimeraplast Constructs;
Co-Suppression;
Transcriptional Silencing; and
Other Methods of Gene Expression.

C.1 Antisense

In some instances it is desirable to suppress expression of an endogenous or exogenous gene. A well-known instance is the FLAVOR-SAVOR™ tomato, in which the gene encoding ACC synthase is inactivated by an antisense approach, thus delaying softening of the fruit after ripening. See for example, U.S. Patent No. 5,859,330; U.S. Patent No. 5,723,766; Oeller, et al, *Science,* 254:437-439(1991); and Hamilton et al, *Nature,* 346:284-287 (1990). Also, timing of flowering can be controlled by suppression of the *FLOWERING LOCUS C* (*FLC*); high levels of this transcript are associated with late flowering, while absence of *FLC* is associated with early flowering (S.D. Michaels et al., *Plant Cell* 11:949 (1999). Also, the transition of apical meristem from production of leaves with associated shoots to flowering is regulated by *TERMINAL FLOWER1, APETALA1* and *LEAFY.* Thus, when it is desired to induce a transition from shoot production to flowering, it is desirable to suppress *TFL1* expression (S.J. Liljegren, *Plant Cell* 11:1007 (1999)). As another instance, arrested ovule development and female sterility result from suppression of the ethylene forming enzyme but can be reversed by application of ethylene (D. De Martinis et al., *Plant Cell* 11:1061 (1999)). The ability to manipulate female fertility of plants is useful in increasing fruit production and creating hybrids.

In the case of polynucleotides used to inhibit expression of an endogenous gene, the introduced sequence need not be perfectly identical to a sequence of the target endogenous gene. The introduced polynucleotide sequence will typically be at least substantially identical to the target endogenous sequence.

Some polynucleotide SDFs in Table 1 represent sequences that are expressed in corn,wheat, rice, soybean *Arabidopsis* and/or other plants. Thus the invention includes using these sequences to generate antisense constructs to inhibit translation and/or degradation of transcripts of said SDFs, typically in a plant cell.

To accomplish this, a polynucleotide segment from the desired gene that can hybridize to the mRNA expressed from the desired gene (the "antisense segment") is operably linked to a promoter such that the antisense strand of RNA will be transcribed when the construct is present in a host cell. A regulated promoter can be used in the construct to control transcription of the antisense segment so that transcription occurs only under desired circumstances.

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The antisense segment to be introduced generally will be substantially identical to at least a fragment of the endogenous gene or genes to be repressed. The sequence, however, need not be perfectly identical to inhibit expression. Further, the antisense product may hybridize to the untranslated region instead of or in addition to the coding sequence of the gene. The vectors of the present invention can be designed such that the inhibitory effect applies to other proteins within a family of genes exhibiting homology or substantial homology to the target gene.

For antisense suppression, the introduced antisense segment sequence also need not be full length relative to either the primary transcription product or the fully processed mRNA. Generally, a higher percentage of sequence identity can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective. Normally, a sequence of between about 30 or 40 nucleotides and the full length of the transcript canbe used, though a sequence of at least about 100 nucleotides is preferred, a sequence of at least about 200 nucleotides is more preferred, and a sequence of at least about 500 nucleotides is especially preferred.

C.2. Ribozymes

It is also contemplated that gene constructs representing ribozymes and based on the SDFs in TABLE 1 are an object of the invention. Ribozymes can also be used to inhibit expression of genes by suppressing the translation of the mRNA into a polypeptide. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs.

A number of classes of ribozymes have been identified. One class of ribozymes is derived from a number of small circular RNAs, which are capable of self-cleavage and replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus, solanum nodiflorum mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Haseloff et al. *Nature,* 334:585 (1988).

Like the antisense constructs above, the ribozyme sequence fragment necessary for pairing need not be identical to the target nucleotides to be cleaved, nor identical to the sequences in TABLE 1. Ribozymes may be constructed by combining the ribozyme sequence and some fragment of the target gene which would allow recognition of the target gene mRNA by the resulting ribozyme molecule. Generally, the sequence in the ribozyme capable of binding to the target sequence exhibits a percentage of sequence identity with at least 80%, preferably with at least 85%, more preferably with at least 90% and most preferably with at least 95%, even more preferably, with at least 96%, 97%, 98% or 99% sequence identity to some fragment of a sequence in TABLE 1 or the complement thereof. The ribozyme can be equally effective in inhibiting mRNA translation by cleaving either in the untranslated or coding regions. Generally, a higher percentage of sequence identity can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective.

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C.3. Chimeraplasts

The SDFs of the invention, such as those described by Table 1, can also be used to construct chimeraplasts that can be introduced into a cell to produce at least one specific nucleotide change in a sequence corresponding to the SDF of the invention. A chimeraplast is an oligonucleotide comprising DNA and/or RNA that specifically hybridizes to a target region in a manner which creates a mismatched base-pair. This mismatched base-pair signals the cell's repair enzyme machinery which acts on the mismatched region resulting in the replacement, insertion or deletion of designated nucleotide(s). The altered sequence is then expressed by the cell's normal cellular mechanisms. Chimeraplasts can be designed to repair mutant genes, modify genes, introduce site-specific mutations, and/or act to interrupt or alter normal gene function (US Pat. Nos. 6,010,907 and 6,004,804; and PCT Pub. No. WO99/58723 and WO99/07865).

C.4. Sense Suppression

The SDFs of Table 1 of the present invention are also useful to modulate gene expression by sense suppression. Sense suppression represents another method of gene suppression by introducing at least one exogenous copy or fragment of the endogenous sequence to be suppressed.

Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter into the chromosome of a plant or by a self-replicating virus has been shown to be an effective means by which to induce degradation of mRNAs of target genes. For an example of the use of this method to modulate expression of endogenous genes *see*, Napoli et al., *The Plant Cell* 2:279 (1990), and U.S. Patents Nos. 5,034,323, 5,231,020, and 5,283,184. Inhibition of expression may require some transcription of the introduced sequence.

For sense suppression, the introduced sequence generally will be substantially identical to the endogenous sequence intended to be inactivated. The minimal percentage of sequence identity will typically be greater than about 65%, but a higher percentage of sequence identity might exert a more effective reduction in the level of normal gene products. Sequence identity of more than about 80% is preferred, though about 95% to absolute identity would be most preferred. As with antisense regulation, the effect would likely apply to any other proteins within a similar family of genes exhibiting homology or substantial homology to the suppressing sequence.

C.5. Transcriptional Silencing

The nucleic acid sequences of the invention, including the SDFs of Table 1, and fragments thereof, contain sequences that can be inserted into the genome of an organism resulting in transcriptional silencing. Such regulatory sequences need not be operatively linked to coding sequences to modulate transcription of a gene. Specifically, a promoter sequence without any other element of a gene can be introduced into a genome to transcriptionally silence an endogenous gene (see, for example, Vaucheret, H et al. (1998) The Plant Journal 16: 651-659). As another example, triple helices can be formed using oligonucleotides based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The oligonucleotide can be delivered to the host cell and can bind to the promoter in the genome to form a triple helix and prevent transcription. An oligonucleotide of interest is one that can bind to the promoter and block binding of a transcription factor to the promoter. In such a case, the oligonucleotide can be complementary to the sequences of the promoter that interact with transcription binding factors.

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C.6. Other Methods to Inhibit Gene Expression

Yet another means of suppressing gene expression is to insert a polynucleotide into the gene of interest to disrupt transcription or translation of the gene.

Low frequency homologous recombination can be used to target a polynucleotide insert to a gene by flanking the polynucleotide insert with sequences that are substantially similar to the gene to be disrupted. Sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto can be used for homologous recombination.

In addition, random insertion of polynucleotides into a host cell genome can also be used to disrupt the gene of interest. Azpiroz-Leehan et al., *Trends in Genetics* 13:152 (1997). In this method, screening for clones from a library containing random insertions is preferred to identifying those that have polynucleotides inserted into the gene of interest. Such screening can be performed using probes and/or primers described above based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The screening can also be performed by selecting clones or R_1 plants having a desired phenotype.

I.D. Methods of Functional Analysis

The constructs described in the methods under I.C. above can be used to determine the function of the polypeptide encoded by the gene that is targeted by the constructs.

Down-regulating the transcription and translation of the targeted gene in the host cell or organisms, such as a plant, may produce phenotypic changes as compared to a wild-type cell or organism. In addition, *in vitro* assays can be used to determine if any biological activity, such as calcium flux, DNA transcription, nucleotide incorporation, etc., are being modulated by the down-regulation of the targeted gene.

Coordinated regulation of sets of genes, e.g., those contributing to a desired polygenic trait, is sometimes necessary to obtain a desired phenotype. SDFs of the invention representing transcription activation and DNA binding domains can be assembled into hybrid transcriptional activators. These hybrid transcriptional activators can be used with their corresponding DNA elements (i.e., those bound by the DNA-binding SDFs) to effect coordinated expression of desired genes (J.J. Schwarz et al., *Mol. Cell. Biol.* 12:266 (1992), A. Martinez et al., *Mol. Gen. Genet.* 261:546 (1999)).

The SDFs of the invention can also be used in the two-hybrid genetic systems to identify networks of protein-protein interactions (L. McAlister-Henn et al., *Methods* 19:330 (1999), J.C. Hu et al., *Methods* 20:80 (2000), M. Golovkin et al., *J. Biol. Chem.* 274:36428 (1999), K. Ichimura et al., *Biochem. Biophys. Res. Comm.* 253:532 (1998)). The SDFs of the invention can also be used in various expression display methods to identify important protein-DNA interactions (e.g. B. Luo et al., *J. Mol. Biol.* 266:479 (1997)).

I.E. Promoters

The SDFs of the invention are also useful as structural or regulatory sequences in a construct for modulating the expression of the corresponding gene in a plant or other organism, *e.g.* a symbiotic bacterium. For example, promoter sequences associated to SDFs of Table 1 of the present invention can be useful in directing expression of coding sequences either as constitutive promoters or to direct expression in particular cell types, tissues, or organs or in response to environmental stimuli.

With respect to the SDFs of the present invention a promoter is likely to be a relatively small portion of a genomic DNA (gDNA) sequence located in the first 2000 nucleotides upstream from an initial exon identified in a gDNA sequence or initial "ATG" or methionine codon or translational start site in a corresponding cDNA sequence. Such promoters are more likely to be found in the first 1000 nucleotides upstream of an initial ATG or methionine codon or translational start site of a cDNA sequence corresponding to a gDNA sequence. In particular, the promoter is usually located upstream of the transcription start site. The fragments of a particular gDNA sequence that function as elements of a promoter in a plant cell will preferably be found to hybridize to gDNA sequences presented and described in Table 1 at medium or high stringency, relevant to the length of the probe and its base composition.

Promoters are generally modular in nature. Promoters can consist of a basal promoter that functions as a site for assembly of a transcription complex comprising an RNA polymerase, for example RNA polymerase II. A typical transcription complex will include additional factors such as $TF_{II}B$, $TF_{II}D$, and $TF_{II}E$. Of these, $TF_{II}D$ appears to be the only one to bind DNA directly. The promoter might also contain one or more enhancers and/or suppressors that function as binding sites for additional transcription factors that have the function of modulating the level of transcription with respect to tissue specificity and of transcriptional responses to particular environmental or nutritional factors, and the like.

Short DNA sequences representing binding sites for proteins can be separated from each other by intervening sequences of varying length. For example, within a particular functional module, protein binding sites may be constituted by regions of 5 to 60, preferably 10 to 30, more preferably 10 to 20 nucleotides. Within such binding sites, there are typically 2 to 6 nucleotides that specifically contact amino acids of the nucleic acid binding protein. The protein binding sites are usually separated from each other by 10 to several hundred nucleotides, typically by 15 to 150 nucleotides, often by 20 to 50 nucleotides. DNA binding sites in promoter elements often display dyad symmetry in their sequence. Often elements binding several different proteins, and/or a plurality of sites that bind the same protein, will be combined in a region of 50 to 1,000 basepairs.

Elements that have transcription regulatory function can be isolated from their corresponding endogenous gene, or the desired sequence can be synthesized, and recombined in constructs to direct expression of a coding region of a gene in a desired tissue-specific, temporal-specific or other desired manner of inducibility or suppression. When hybridizations are performed to identify or isolate elements of a promoter by hybridization to the long sequences presented in TABLE 1, conditions are adjusted to account for the above-described nature of promoters. For example short probes, constituting the element sought, are preferably used under low temperature and/or high salt conditions. When long probes, which might include several promoter elements are used, low to medium stringency conditions are preferred when hybridizing to promoters across species.

If a nucleotide sequence of an SDF, or part of the SDF, functions as a promoter or fragment of a promoter, then nucleotide substitutions, insertions or deletions that do not substantially affect the binding of relevant DNA binding proteins would be considered equivalent to the exemplified nucleotide sequence. It is envisioned that there are instances where it is desirable to decrease the binding of relevant DNA binding proteins to silence or down-regulate a promoter, or conversely to increase the binding of relevant DNA binding proteins to enhance or up-regulate a promoter and vice versa. In such instances, polynucleotides representing changes to the nucleotide sequence of the DNA-protein contact region by insertion of additional nucleotides, changes to identity of relevant nucleotides, including use of chemically-modified bases, or deletion of one or more nucleotides are considered encompassed by the present invention. In addition, fragments of the promoter sequences described by Table 1 and variants thereof can be fused with other promoters or fragments to facilitate transcription and/or transcription in specific type of cells or under specific conditions.

Promoter function can be assayed by methods known in the art, preferably by measuring activity of a reporter gene operatively linked to the sequence being tested for promoter function. Examples of reporter genes include those encoding luciferase, green fluorescent protein, GUS, neo, cat and bar.

I.F. UTRs and Junctions

Polynucleotides comprising untranslated (UTR) sequences and intron/exon junctions are also within the scope of the invention. UTR sequences include introns and 5' or 3' untranslated regions (5' UTRs or 3' UTRs). Fragments of the sequences shown in TABLE 1 can comprise UTRs and intron/exon junctions.

These fragments of SDFs, especially UTRs, can have regulatory functions related to, for example, translation rate and mRNA stability. Thus, these fragments of SDFs can be isolated for use as elements of gene constructs for regulated production of polynucleotides encoding desired polypeptides.

Introns of genomic DNA segments might also have regulatory functions. Sometimes regulatory elements, especially transcription enhancer or suppressor elements, are found within introns. Also, elements related to stability of heteronuclear RNA and efficiency of splicing and of transport to the cytoplasm for translation can be found in intron elements. Thus, these segments can also find use as elements of expression vectors intended for use to transform plants.

Just as with promoters UTR sequences and intron/exon junctions can vary from those shown in TABLE 1. Such changes from those sequences preferably will not affect the regulatory activity of the UTRs or intron/exon junction sequences on expression, transcription, or translation unless selected to do so. However, in some instances, down- or up-regulation of such activity may be desired to modulate traits or phenotypic or *in vitro* activity.

I.G. Coding Sequences

Isolated polynucleotides of the invention can include coding sequences that encode polypeptides comprising an amino acid sequence encoded by sequences in TABLE 1 or an amino acid sequence presented in TABLE 1.

A nucleotide sequence encodes a polypeptide if a cell (or a cell free *in vitro* system) expressing that nucleotide sequence produces a polypeptide having the recited amino acid sequence when the nucleotide sequence is transcribed and the primary transcript is subsequently processed and translated by a host cell (or a cell free *in vitro* system) harboring the nucleic acid. Thus, an isolated nucleic acid that encodes a particular amino acid sequence can be a genomic sequence comprising exons and introns or a cDNA sequence that represents the product of splicing thereof. An isolated nucleic acid encoding an amino acid sequence also encompasses heteronuclear RNA, which contains sequences that are spliced out during expression, and mRNA, which lacks those sequences.

Coding sequences can be constructed using chemical synthesis techniques or by isolating coding sequences or by modifying such synthesized or isolated coding sequences as described above.

In addition to coding sequences encoding the polypeptide sequences of TABLE 1, which are native to corn, *Arabidopsis*, soybean, rice, wheat, and other plants the isolated polynucleotides can be polynucleotides that encode variants, fragments, and fusions of those native proteins. Such polypeptides are described below in part II.

In variant polynucleotides generally, the number of substitutions, deletions or insertions is preferably less than 20%, more preferably less than 15%; even more preferably less than 10%, 5%, 3% or 1% of the number of nucleotides comprising a particularly exemplified sequence. It is generally expected that non-degenerate nucleotide sequence changes that result in 1 to 10, more preferably 1 to 5 and most preferably 1 to 3 amino acid insertions, deletions or substitutions will not greatly affect the function of an encoded polypeptide. The most preferred embodiments are those wherein 1 to 20, preferably 1 to 10, most preferably 1 to 5 nucleotides are added to, deleted from and/or substituted in the sequences specifically disclosed in TABLE 1.

Insertions or deletions in polynucleotides intended to be used for encoding a polypeptide preferably preserve the reading frame. This consideration is not so important in instances when the polynucleotide is intended to be used as a hybridization probe.

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II. Polypeptides and Proteins

IIA. Native polypeptides and proteins

Polypeptides within the scope of the invention include both native proteins as well as variants, fragments, and fusions thereof. Polypeptides of the invention are those encoded by any of the six reading frames of sequences shown in TABLE 1, preferably encoded by the three frames reading in the 5' to 3' direction of the sequences as shown.

Native polypeptides include the proteins encoded by the sequences shown in TABLE 1. Such native polypeptides include those encoded by allelic variants.

Polypeptide and protein variants will exhibit at least 75% sequence identity to those native polypeptides of TABLE 1. More preferably, the polypeptide variants will exhibit at least 85% sequence identity; even more preferably, at least 90% sequence identity; more preferably at least 95%, 96%, 97%, 98%, or 99% sequence identity. Fragments of polypeptide or fragments of polypeptides will exhibit similar percentages of sequence identity to the relevant fragments of the native polypeptide. Fusions will exhibit a similar percentage of sequence identity in that fragment of the fusion represented by the variant of the native peptide.

Furthermore, polypeptide variants will exhibit at least one of the functional properties of the native protein. Such properties include, without limitation, protein interaction, DNA interaction, biological activity, immunological activity, receptor binding, signal transduction, transcription activity, growth factor activity, secondary structure, three-dimensional structure, etc. As to properties related to *in vitro* or *in vivo* activities, the variants preferably exhibit at least 60% of the activity of the native protein; more preferably at least 70%, even more preferably at least 80%, 85%, 90% or 95% of at least one activity of the native protein.

One type of variant of native polypeptides comprises amino acid substitutions, deletions and/or insertions. Conservative substitutions are preferred to maintain the function or activity of the polypeptide.

Within the scope of percentage of sequence identity described above, a polypeptide of the invention may have additional individual amino acids or amino acid sequences inserted into the polypeptide in the middle thereof and/or at the N-terminal and/or C-terminal ends thereof. Likewise, some of the amino acids or amino acid sequences may be deleted from the polypeptide.

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A.1 Antibodies

Isolated polypeptides can be utilized to produce antibodies. Polypeptides of the invention can generally be used, for example, as antigens for raising antibodies by known techniques. The resulting antibodies are useful as reagents for determining the distribution of the antigen protein within the tissues of a plant or within a cell of a plant. The antibodies are also useful for examining the production level of proteins in various tissues, for example in a wild-type plant or following genetic manipulation of a plant, by methods such as Western blotting.

Antibodies of the present invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the polypeptides of the invention are first used to immunize a suitable animal, such as a mouse, rat, rabbit, or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies as detection reagents. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 μg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by *in vitro* immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization.

Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating the blood at 4°C for 2-18 hours. The serum is recovered by centrifugation (e.g., 1,000xg for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the method of Kohler and Milstein, *Nature* 256: 495 (1975), or modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells can be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate, or well, coated with the protein antigen. B-cells producing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected Mab-secreting hybridomas are then cultured either *in vitro* (*e.g.*, in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

Other methods for sustaining antibody-producing B-cell clones, such as by EBV transformation, are known.

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TNB) to a blue pigment, quantifiable with a spectrophotometer.

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A.2 In Vitro Applications of Polypeptides

Some polypeptides of the invention will have enzymatic activities that are useful *in vitro*. For example, the soybean trypsin inhibitor (Kunitz) family is one of the numerous families of proteinase inhibitors. It comprises plant proteins which have inhibitory activity against serine proteinases from the trypsin and subtilisin families, thiol proteinases and aspartic proteinases. Thus, these peptides find *in vitro* use in protein purification protocols and perhaps in therapeutic settings requiring topical application of protease inhibitors.

Delta-aminolevulinic acid dehydratase (EC 4.2.1.24) (ALAD) catalyzes the second step in the biosynthesis of heme, the condensation of two molecules of 5-aminolevulinate to form porphobilinogen and is also involved in chlorophyll biosynthesis(Kaczor et al. (1994) Plant Physiol. 1-4: 1411-7; Smith (1988) Biochem. J. 249: 423-8; Schneider (1976) Z. naturforsch. [C] 31: 55-63). Thus, ALAD proteins can be used as catalysts in synthesis of heme derivatives. Enzymes of biosynthetic pathways generally can be used as catalysts for *in vitro* synthesis of the compounds representing products of the pathway.

Polypeptides encoded by SDFs of the invention can be engineered to provide purification reagents to identify and purify additional polypeptides that bind to them. This allows one to identify proteins that function as multimers or elucidate signal transduction or metabolic pathways. In the case of DNA binding proteins, the polypeptide can be used in a similar manner to identify the DNA determinants of specific binding (S. Pierrou et al., *Anal.*

Biochem. 229:99 (1995), S. Chusacultanachai et al., *J. Biol. Chem.* 274:23591 (1999), Q. Lin et al., *J. Biol. Chem.* 272:27274 (1997)).

II.B . POLYPEPTIDE VARIANTS , FRAGMENTS, AND FUSIONS

Generally, variants , fragments, or fusions of the polypeptides encoded by the SDFs of the invention can exhibit at least one of the activities of the identified domains and/or related polypeptides described in Table 1 corresponding to the SDF of interest.

II.B .(1) Variants

A type of variant of the native polypeptides comprises amino acid substitutions. Conservative substitutions, described above (see II.), are preferred to maintain the function or activity of the polypeptide. Such substitutions include conservation of charge, polarity, hydrophobicity, size, etc. For example, one or more amino acid residues within the sequence can be substituted with another amino acid of similar polarity that acts as a functional equivalent, for example providing a hydrogen bond in an enzymatic catalysis. Substitutes for an amino acid within an exemplified sequence are preferably made among the members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Within the scope of percentage of sequence identity described above, a polypeptide of the invention may have additional individual amino acids or amino acid sequences inserted into the polypeptide in the middle thereof and/or at the N-terminal and/or C-terminal ends thereof. Likewise, some of the amino acids or amino acid sequences may be deleted from the polypeptide. Amino acid substitutions may also be made in the sequences; conservative substitutions being preferred.

One preferred class of variants are those that comprise (1) the domain of an encoded polypeptide and/or (2) residues conserved between the encoded polypeptide and related polypeptides. For this class of variants, the encoded polypeptide sequence is changed by insertion, deletion, or substitution at positions flanking the domain and/or conserved residues.

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Another class of variants includes those that comprise an encoded polypeptide sequence that is changed in the domain or conserved residues by a conservative substitution.

Yet another class of variants includes those that lack one of the *in vitro* activities, or structural features of the encoded polypeptides. One example is polypeptides or proteins produced from genes comprising dominant negative mutations. Such a variant may comprise an encoded polypeptide sequence with non-conservative changes in a particular domain or group of conserved residues.

II.A.(2) FRAGMENTS

Fragments of particular interest are those that comprise a domain identified for a polypeptide encoded by an SDF of the instant invention and variants thereof. Also, fragments that comprise at least one region of residues conserved between an SDF encoded polypeptide and its related polypeptides are of great interest. Fragments are sometimes useful as polypeptides corresponding to genes comprising dominant negative mutations are.

II.A.(3)FUSIONS

Of interest are chimeras comprising (1) a fragment of the SDF encoded polypeptide or variants thereof of interest and (2) a fragment of a polypeptide comprising the same domain. For example, an AP2 helix encoded by a SDF of the invention fused to second AP2 helix from ANT protein, which comprises two AP2 helices. The present invention also encompasses fusions of SDF encoded polypeptides, variants, or fragments thereof fused with related proteins or fragments thereof.

DEFINITION OF DOMAINS

The polypeptides of the invention may possess identifying domains. In addition, the domains within the SDF encoded polypeptide can be defined by the region that exhibits at least 70% sequence identity with the consensus sequences listed in the detailed description below of each of the domains.

The majority of the protein domain descriptions given below are obtained from Prosite,
(http//www.expasy.ch/prosite/), and Pfam,
(http//pfam.wustl.edu/browse.shtml).

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1. (AAA) AAA-protein family signature

A large family of ATPases has been described [1 to 5] whose key feature is that they share a conserved region of about 220 amino acids that contains anATP-binding site. This family is now called AAA, for 'A'TPases 'A'ssociated with diverse cellular 'A'ctivities. The proteins that belong to this family either contain one or two AAA domains. Proteins containing two AAA domains:

- Mammalian and drosophila NSF (N-ethylmaleimide-sensitive fusion protein) and the fungal homolog, SEC18. These proteins are involved in intracellular transport between the endoplasmic reticulum and Golgi, as well as between different Golgi cisternae.
- Mammalian transitional endoplasmic reticulum ATPase (previously known as p97 or VCP) which is involved in the transfer of membranes from the endoplasmic reticulum to the golgi apparatus. This protein forms a ring-shaped homooligomer composed of six subunits. The yeast homolog is CDC48 and it may play a role in spindle pole proliferation.
- Yeast protein PAS1, essential for peroxisome assembly and the related protein PAS1 from Pichia pastoris.
- Yeast protein AFG2.
- Sulfolobus acidocaldarius protein SAV and Halobacterium salinarium cdcH which may be part of a transduction pathway connecting light to cell division.

Proteins containing a single AAA domain:

- Escherichia coli and other bacteria ftsH (or hflB) protein. FtsH is an ATP-dependent zinc metallopeptidase that seems to degrade the heat-shock sigma-32 factor.

It is an integral membrane protein with a large cytoplasmic C-terminal domain that contain both the AAA and the protease domains.

- Yeast protein YME1, a protein important for maintaining the integrity of the mitochondrial compartment. YME1 is also a zinc-dependent protease.
- Yeast protein AFG3 (or YTA10). This protein also seems to contain a AAA domain followed by a zinc-dependent protease domain.

Subunits from the regulatory complex of the 26S proteasome [6] which is involved in the ATP-dependent degradation of ubiquitinated proteins:

a) Mammalian subunit 4 and homologs in other higher eukaryotes, in yeast (gene YTA5) and fission yeast (gene mts2).

b) Mammalian subunit 6 (TBP7) and homologs in other higher eukaryotes and in yeast (gene YTA2).

c) Mammalian subunit 7 (MSS1) and homologs in other higher eukaryotes and in yeast (gene CIM5 or YTA3).

d) Mammalian subunit 8 (P45) and homologs in other higher eukaryotes and in yeast (SUG1 or CIM3 or TBY1) and fission yeast (gene let1).

Other probable subunits such as human TBP1 which seems to influences HIV gene expression by interacting with the virus tat transactivator protein and yeast YTA1 and YTA6.

- Yeast protein BCS1, a mitochondrial protein essential for the expression of the Rieske iron-sulfur protein.
- Yeast protein MSP1, a protein involved in intramitochondrial sorting of proteins.
- Yeast protein PAS8, and the corresponding proteins PAS5 from Pichia pastoris and PAY4 from Yarrowia lipolytica.
- Mouse protein SKD1 and its fission yeast homolog (SpAC2G11.06).
- Caenorhabditis elegans meiotic spindle formation protein mei-1.
- Yeast protein SAP1.
- Yeast protein YTA7.
- Mycobacterium leprae hypothetical protein A2126A.

It is proposed that, in general, the AAA domains in these proteins act as ATP-dependent protein clamps [5]. In addition to the ATP-binding 'A' and 'B' motifs, which are located in the N-terminal half of this domain, there is a highly conserved region located in the central part of the domain which was used to develop a signature pattern.

Consensus pattern: [LIVMT]-x-[LIVMT]-[LIVMF]-x-[GATMC]-[ST]-[NS]-x(4)-[LIVM]-D-x-A-[LIFA]-x-R

[1] Froehlich K.-U., Fries H.W., Ruediger M., Erdmann R., Botstein D., Mecke D. J. Cell Biol. 114:443-453(1991).

[2] Erdmann R., Wiebel F.F., Flessau A., Rytka J., Beyer A., Froehlich K.-U., Kunau W.-H. Cell 64:499-510(1991).

[3] Peters J.-M., Walsh M.J., Franke W.W. EMBO J. 9:1757-1767(1990).

[4] Kunau W.-H., Beyer A., Goette K., Marzioch M., Saidowsky J., Skaletz-Rorowski A., Wiebel F.F. Biochimie 75:209-224(1993).

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[5] Confalonieri F., Duguet M. BioEssays 17:639-650(1995).[6] Hilt W., Wolf D.H. Trends Biochem. Sci. 21:96-102(1996).

2. ABC Membrane (ABC transporter transmembrane region). This family represents a unit of six transmembrane helices. Many members of the ABC transporter family (ABC_tran)have two such regions. See also descriptions of ABC Tran, below, and ABC2 membrane, above.

3. (ABC Tran) ABC transporters family signature. On the basis of sequence similarities a family of related ATP-bindingproteins has been characterized [1 to 5]. These proteins are associated with avariety of distinct biological processes in both prokaryotes and eukaryotes, but a majority of them are involved in active transport of small hydrophilic molecules across the cytoplasmic membrane. All these proteins share a conserved domain of some two hundred amino acid residues, which includes an ATP-binding site. These proteins are collectively known as ABC transporters. Proteins known to belong to this family are listed below (references are only provided for recently determined sequences).In prokaryotes: - Active transport systems components: alkylphosphonate uptake(phnC/phnK/ phnL); arabinose (araG); arginine (artP); dipeptide (dciAD;dppD/dppF); ferric enterobactin (fepC); ferrichrome (fhuC); galactoside (mglA); glutamine (glnQ); glycerol-3-phosphate (ugpC); glycine betaine/L-proline (proV); glutamate/aspatate (gltL); histidine (hisP); iron(III) (sfuC), iron(III) dicitrate (fecE); lactose (lacK); leucine/isoleucine/valine (braF/braG;livF/livG); maltose (malK); molybdenum (modC); nickel (nikD/ nikE); oligopeptide (amiE/amiF;oppD/oppF); peptide (sapD/sapF); phosphate (pstB); putrescine (potG); ribose (rbsA); spermidine/putrescine (potA); sulfate (cysA); vitamin B12 (btuD). - Hemolysin/leukotoxin export proteins hlyB, cyaB and lktB. - Colicin V export protein cvaB. - Lactococcin export protein lcnC [6]. - Lantibiotic transport proteins nisT (nisin) and spaT (subtilin). - Extracellular proteases B and C export protein prtD. - Alkaline protease secretion protein aprD. - Beta-(1,2)-glucan export proteins chvA and ndvA. - Haemophilus influenzae capsule-polysaccharide export protein bexA. - Cytochrome c biogenesis proteins ccmA (also known as cycV and helA). - Polysialic acid transport protein kpsT. - Cell division associated ftsE protein (function unknown). - Copper processing protein nosF from Pseudomonas stutzeri. - Nodulation protein nodI from Rhizobium (function unknown). - Escherichia coli proteins cydC and cydD. - Subunit A of the ABC excision nuclease (gene uvrA). -

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Erythromycin resistance protein from Staphylococcus epidermidis (gene msrA). - Tylosin resistance protein from Streptomyces fradiae (gene tlrC) [7]. - Heterocyst differentiation protein (gene hetA) from Anabaena PCC 7120. - Protein P29 from Mycoplasma hyorhinis, a probable component of a high affinity transport system. - yhbG, a putative protein whose gene is linked with ntrA in many bacteria such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas putida, Rhizobium meliloti and Thiobacillus ferrooxidans. - Escherichia coli and related bacteria hypothetical proteins yabJ, yadG, yagC, ybbA, ycjW, yddA, yehX, yejF, yheS, yhiG, yhiH, yjcW, yjjK, yojI, yrbF and ytfR.In eukaryotes: - The multidrug transporters (Mdr) (P-glycoprotein), a family of closely related proteins which extrude a wide variety of drugs out of the cell (for a review see [8]). - Cystic fibrosis transmembrane conductance regulator (CFTR), which is most probably involved in the transport of chloride ions. - Antigen peptide transporters 1 (TAP1, PSF1, RING4, HAM-1, mtp1) and 2 (TAP2, PSF2, RING11, HAM-2, mtp2), which are involved in the transport of antigens from the cytoplasm to a membrane-bound compartment for association with MHC class I molecules. - 70 Kd peroxisomal membrane protein (PMP70). - ALDP, a peroxisomal protein involved in X-linked adrenoleukodystrophy [9]. - Sulfonylurea receptor [10], a putative subunit of the B-cell ATP-sensitive potassium channel. - Drosophila proteins white (w) and brown (bw), which are involved in the import of ommatidium screening pigments. - Fungal elongation factor 3 (EF-3). - Yeast STE6 which is responsible for the export of the a-factor pheromone. - Yeast mitochondrial transporter ATM1. - Yeast MDL1 and MDL2. - Yeast SNQ2. - Yeast sporidesmin resistance protein (gene PDR5 or STS1 or YDR1). - Fission yeast heavy metal tolerance protein hmt1. This protein is probably involved in the transport of metal-bound phytochelatins. - Fission yeast brefeldin A resistance protein (gene bfr1 or hba2). - Fission yeast leptomycin B resistance protein (gene pmd1). - mbpX, a hypothetical chloroplast protein from Liverwort. - Prestalk-specific protein tagB from slime mold. This protein consists of two domains: a N-terminal subtilase catalytic domain and a C-terminal ABC transporter domain.As a signature pattern for this class of proteins, a conserved region which is located between the 'A' and the 'B' motifs of the ATP-binding site was used.

Consensus pattern: [LIVMFYC]-[SA]-[SAPGLVFYKQH]-G-[DENQMW]-[KRQASPCLIMFW]-[KRNQSTAVM]-[KRACLVM]-[LIVMFYPAN]-{PHY}-[LIVMFW]-[SAGCLIVP]-{FYWHP}-{KRHP}-[LIVMFYWSTA] The ATP-binding region is duplicated in araG, mdl, msrA, rbsA, tlrC, uvrA, yejF, Mdr's, CFTR, pmd1 and in EF-3. In some of those proteins, the above pattern only detect one of the two copies of the domain. The proteins belonging to this family also contain one or two copies of the ATP-binding motifs 'A' and 'B'.

[1] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg. Biomembr. 22:571-592(1990).
[2] Higgins C.F., Gallagher M.P., Mimmack M.M., Pearce S.R. BioEssays 8:111-116(1988).
[3] Higgins C.F., Hiles I.D., Salmond G.P.C., Gill D.R., Downie J.A., Evans I.J., Holland I.B., Gray L., Buckels S.D., Bell A.W., Hermodson M.A. Nature 323:448-450(1986).
[4] Doolittle R.F., Johnson M.S., Husain I., van Houten B., Thomas D.C., Sancar A. Nature 323:451-453(1986).
[5] Blight M.A., Holland I.B. Mol. Microbiol. 4:873-880(1990).
[6] Stoddard G.W., Petzel J.P., van Belkum M.J., Kok J., McKay L.L. Appl. Environ. Microbiol. 58:1952-1961(1992).
[7] Rosteck P.R. Jr., Reynolds P.A., Hershberger C.L. Gene 102:27-32(1991).
[8] Gottesman M.M., Pastan I. J. Biol. Chem. 263:12163-12166(1988).
[9] Valle D., Gaertner J. Nature 361:682-683(1993).
[10] Aguilar-Bryan L., Nichols C.G., Wechsler S.W., Clement J.P. IV, Boyd A.E. III, Gonzalez G., Herrera-Sosa H., Nguy K., Bryan J., Nelson D.A. Science 268:423-426(1995).

4. (ACBP)
Acyl-CoA-binding protein signature

Acyl-CoA-binding protein (ACBP) is a small (10 Kd) protein that binds medium- and long-chain acyl-CoA esters with very high affinity and may function as an intracellular carrier of acyl-CoA esters [1]. ACBP is also known as diazepam binding inhibitor (DBI) or endozepine (EP) because of its ability to displace diazepam from the benzodiazepine (BZD) recognition site located on the GABA type A receptor. It is therefore possible that this protein also acts as a neuropeptide to modulate the action of the GABA receptor [2].ACBP is a highly conserved protein of about 90 residues that has been so far found in vertebrates, insects and yeast. ACBP is also related to the N-terminal section of a probable transmembrane protein of unknown function whichhas been found in mammals. As a signature pattern, the region that corresponds to residues 19 to 37 in mammalian ACBP was selected.

Consensus pattern: P-[STA]-x-[DEN]-x-[LIVMF]-x(2)-[LIVMFY]-Y-[GSTA]-x-[FY]-K- Q-[STA](2)-x-G-

[1] Rose T.M., Schultz E.R., Todaro G.J. Proc. Natl. Acad. Sci. U.S.A. 89:11287-11291(1992).
[2] Costa E., Guidotti A. Life Sci. 49:325-344(1991).

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5. (AIRS)
AIR synthase related proteins

This family includes Hydrogen expression/formation protein HypE, AIR synthases, FGAM synthase and selenide, water dikinase.

6. (AMP-binding)
Putative AMP-binding domain signature

It has been shown [1 to 5] that a number of prokaryotic and eukaryotic enzymes which all probably act via an ATP-dependent covalent binding of AMP to their substrate, share a region of sequence similarity. These enzymes are: - Insects luciferase (luciferin 4-monooxygenase). Luciferase produces light by catalyzing the oxidation of luciferin in presence of ATP and molecular oxygen. - Alpha-aminoadipate reductase from yeast (gene LYS2). This enzyme catalyzes the activation of alpha-aminoadipate by ATP-dependent adenylation and the reduction of activated alpha-aminoadipate by NADPH. - Acetate--CoA ligase (acetyl-CoA synthetase), an enzyme that catalyzes the formation of acetyl-CoA from acetate and CoA. - Long-chain-fatty-acid--CoA ligase, an enzyme that activates long-chain fatty acids for both the synthesis of cellular lipids and their degradation via beta-oxidation. - 4-coumarate--CoA ligase (4CL), a plant enzyme that catalyzes the formation of 4-coumarate-CoA from 4-coumarate and coenzyme A; the branchpoint reactions between general phenylpropanoid metabolism and pathways leading to various specific end products. - O-succinylbenzoic acid--CoA ligase (OSB-CoA synthetase) (gene menE) [6], a bacterial enzyme involved in the biosynthesis of menaquinone (vitamin K2). - 4-Chlorobenzoate--CoA ligase (EC 6.2.1.-) (4-CBA--CoA ligase) [7], a Pseudomonas enzyme involved in the degradation of 4-CBA. - Indoleacetate--lysine ligase (IAA-lysine synthetase) [8], an enzyme from Pseudomonas syringae that converts indoleacetate to IAA-lysine. - Bile acid-CoA ligase (gene baiB) from Eubacterium strain VPI 12708 [4]. This enzyme catalyzes the ATP-dependent formation of a variety of C-24 bile acid-CoA. - Crotonobetaine/carnitine-CoA ligase (EC 6.3.2.-) from Escherichia coli (gene caiC). - L-(alpha-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACV synthetase) from various fungi (gene acvA or pcbAB). This enzyme catalyzes the first step in the biosynthesis of penicillin and cephalosporin, the formation of ACV from the constituent amino acids. The amino acids seem to be activated by adenylation. It is a protein of around 3700 amino acids that contains three related domains of about 1000 amino acids. - Gramicidin S synthetase I (gene grsA) from Bacillus brevis. This enzyme catalyzes the first step in the biosynthesis of the cyclic antibiotic gramicidin S, the ATP-dependent racemization of phenylalanine - Tyrocidine synthetase I (gene tycA) from Bacillus brevis. The reaction carried out by tycA is identical to that catalyzed by grsA - Gramicidin S synthetase II (gene grsB) from Bacillus brevis. This enzyme is a multifunctional protein that activates and polymerizes proline, valine, ornithine and leucine. GrsB consists of four related domains. - Enterobactin synthetase components E (gene entE) and F (gene entF) from Escherichia coli. These two enzymes are involved in the ATP-dependent activation of respectively 2,3-dihydroxybenzoate and serine during enterobactin (enterochelin) biosynthesis. - Cyclic peptide antibiotic surfactin synthase subunits 1, 2 and 3 from Bacillus subtilis. Subunits 1 and 2 contains three related domains while subunit 3 only contains a single domain. - HC-toxin synthetase (gene HTS1) from Cochliobolus carbonum. This enzyme activates the four amino acids (Pro, L-Ala, D-Ala and 2-amino-9,10-epoxi-8-oxodecanoic acid) that make up HC-toxin, a cyclic tetrapeptide. HTS1 consists of four related domains.There are also some proteins, whose exact function is not yet known, but whichare, very probably, also AMP-binding enzymes. These proteins are: - ORA (octapeptide-repeat antigen), a Plasmodium falciparum protein whose function is not known but which shows a high degree of similarity with the above proteins. - AngR, a Vibrio anguillarum protein. AngR is thought to be a transcriptional activator which modulates the anguibactin (an iron-binding siderophore) biosynthesis gene cluster operon. But it is believed [9], that angR is not a DNA-binding protein, but rather an enzyme involved in the biosynthesis of anguibactin. This conclusion is based on three facts: the presence of the AMP-binding domain; the size of angR (1048 residues), which is far bigger than any bacterial transcriptional protein; and the presence of a probable S-acyl thioesterase immediately downstream of angR. - A hypothetical protein in mmsB 3'region in Pseudomonas aeruginosa. - Escherichia coli hypothetical protein ydiD. - Yeast hypothetical protein YBR041w. - Yeast hypothetical protein YBR222c. - Yeast hypothetical protein YER147c.All these proteins contain a highly conserved region very rich in glycine, serine, and threonine which is followed by a conserved lysine. A parallel can be drawn between this type of domain and the G-x(4)-G-K-[ST] ATP-/GTP-binding 'P-loop' domain or the protein kinases G-x-G-x(2)-[SG]-x(10,20)-KATP-binding domains.

Consensus pattern: [LIVMFY]-x(2)-[STG]-[STAG]-G-[ST]-[STEI]-[SG]-x-[PASLIVM]-[KR] In a majority of cases the residue that follows the Lys at the end of the pattern is a Gly.

[1] Toh H. Protein Seq. Data Anal. 4:111-117(1991).
[2] Smith D.J., Earl A.J., Turner G. EMBO J. 9:2743-2750(1990).
[3] Schroeder J. Nucleic Acids Res. 17:460-460(1989).
[4] Mallonee D.H., Adams J.L., Hylemon P.B. J. Bacteriol. 174:2065-2071(1992).
[5] Turgay K., Krause M., Marahiel M.A. Mol. Microbiol. 6:529-546(1992).
[6] Driscoll J.R., Taber H.W. J. Bacteriol. 174:5063-5071(1992).
[7] Babbitt P.C., Kenyon G.L., Matin B.M., Charest H., Sylvestre M., Scholten J.D., Chang K.-H., Liang P.-H., Dunaway-Mariano D. Biochemistry 31:5594-5604(1992).
[8] Farrell D.H., Mikesell P., Actis L.A., Crosa J.H. Gene 86:45-51(1990).

7. AP2 domain

This 60 amino acid residue domain can bind to DNA [1]. This domain is plant specific. Members of this family are suggested to be related to pyridoxal phosphate-binding domains such as found in aminotran_2 [3]. AP2 domains are also described in Jofuku et al., co-pending U.S. Patent applications 08/700,152, 08/879,827, 08/912,272, 09/026,039.

[1] Ohme-takagi M, Shinshi H; Plant Cell 1995;7:173-182.

[2] Weigel D; Plant Cell 1995;7:388-389.

[3] Mushegian AR, Koonin EV; Genetics 1996;144:817-828.

8. ARID

The ARID domain is an AT-Rich Interaction domain sharing structural homology to DNA replication and repair nucleases and polymerases.

[1] Herrscher RF, Kaplan MH, Lelsz DL, Das C, Scheuermann R, Tucker PW; Genes Dev 1995;9:3067-3082.

[2] Yuan YC, Whitson RH, Liu Q, Itakura K, Chen Y; Nat Struct Biol 1998;5:959-964.

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9. (ATP synt)

ATP synthase gamma subunit signature

ATP synthase (proton-translocating ATPase) (EC 3.6.1.34) [1,2] is a componentof the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), and a catalytic core, called coupling factor CF(1). The former acts as a proton channel; the latter is composed of five subunits, alpha, beta, gamma, delta and epsilon. Subunit gamma is believed to be important in regulating ATPase activity and the flow of protons through the CF(0) complex. The best conserved region of the gamma subunit [3] is its C-terminus which seems to be essential for assembly and catalysis. As a signature pattern to detect ATPase gamma subunits, a14 residue conserved segment where the last amino acid is found one to three residues from the C-terminal extremity was used.

Consensus pattern: [IV]-T-x-E-x(2)-[DE]-x(3)-G-A-x-[SAKR]- Note: Pea chloroplast gamma and two Bacillus species gamma subunits are not detected by this motif.

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).

[2] Senior A.E. Physiol. Rev. 68:177-231(1988).

[3] Miki J., Maeda M., Mukohata Y., Futai M. FEBS Lett. 232:221-226(1988).

10. (ATP Synt A)

Synthase a subunit signature

ATP synthase (proton-translocating ATPase) (EC 3.6.1.34) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria,and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), which acts as a proton channel, and a catalytic core, termed coupling factor CF(1).The CF(0) a subunit, also called protein 6, is a key component of the proton channel; it may play a direct role in translocating protons across the membrane. It is a highly hydrophobic protein that has been predicted to contain 8 transmembrane regions [3].Sequence comparison of a subunits from all available sources reveals very few conserved regions. The best conserved region is located in what is predicted to be the fifth transmembrane domain. This region contains three perfectly conserved residues: an arginine, a leucine and an asparagine. Mutagenesis experiments of ATPase activity. This region was selected as a signature pattern.

Consensus pattern: [STAGN]-x-[STAG]-[LIVMF]-R-L-x-[SAGV]-N-[LIVMT] [R is important for proton translocation]

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).
[2] Senior A.E. Physiol. Rev. 68:177-231(1988).
[3] Lewis M.L., Chang J.A., Simoni R.D. J. Biol. Chem. 265:10541-10550(1990).
[4] Cain B.D., Simoni R.D. J. Biol. Chem. 264:3292-3300(1989).

11. ATP synthase B

Part of the CF(0) (base unit) of the ATP synthase. The base unit is thought to translocate protons through membrane (inner membrane in mitochondria, thylakoid membrane in plants, cytoplasmic membrane in bacteria). The B subunits are thought to interact with the stalk of the CF(1) subunits.

12. (ATP synt C)

ATP synthase c subunit signature

ATP synthase (proton-translocating ATPase) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria,and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), which acts as a proton channel, and a catalytic core, termed coupling factor CF(1).The CF(0) c subunit (also called protein 9, proteolipid, or subunit III) [3,4]is a highly hydrophobic protein of about 8 Kd which has been implicated in the proton-conducting activity of ATPase. Structurally subunit c consist of two long terminal hydrophobic regions, which probably span the membrane, and a central hydrophilic region. N,N'-dicyclohexylcarbodiimide (DCCD) can bind covalently to subunit c and thereby abolish the ATPase activity. DCCD binds to a specific glutamate or aspartate residue which is located in the middle ofthe second hydrophobic region near the C-terminus of the protein. A signature pattern which includes the DCCD-binding residue was derived.

Consensus pattern: [GSTA]-R-[NQ]-P-x(10)-[LIVMFYW](2)-x(3)-[LIVMFYW]-x-[DE] [D or E binds DCCD]

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).
[2] Senior A.E. Physiol. Rev. 68:177-231(1988).
[3] Ivaschenko A.T., Karpenyuk T.A., Ponomarenko S.V. Biokhimiia 56:406-419(1991).
[4] Recipon H., Perasso R., Adoutte A., Quetier F. J. Mol. Evol. 34:292-303(1992).

13. (ATP synt DE)

ATP synthase, Delta/Epsilon chain

Part of the ATP synthase CF(1). These subunits are part of the head unit of the ATP synthase. The subunits are called delta and epsilon in human and metozoan species but in bacterial species the delta (D) subunit is theequivalent to the Oligomycin sensitive subunit (OSCP) in metozoans.

14. (ATP synt ab)

ATP synthase alpha and beta subunits signature

ATP synthase (proton-translocating ATPase) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria,and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), and a catalytic core, called coupling factor CF(1). The former acts as a proton channel; the latter is composed of five subunits, alpha, beta, gamma, delta and epsilon. The sequences of subunits alpha and beta are related and both contain a nucleotide-binding site for ATP and ADP. The beta chain has catalytic activity, while the alpha chain is a regulatory subunit. Vacuolar ATPases [3] (V-ATPases) are responsible for acidifying a variety of intracellular compartments in eukaryotic cells. Like F-ATPases, they are oligomeric complexes of a transmembrane and a catalytic sector. The sequenceof the largest subunit of the catalytic sector (70 Kd) is related to that ofF-ATPase beta subunit, while a 60 Kd subunit, from the same sector, is related to the F-ATPases alpha subunit [4].Archaebacterial membrane-associated ATPases are composed of three subunits.The alpha chain is related to F-ATPases beta chain and the beta chain is related to F-ATPases alpha chain [4].A protein highly similar to F-ATPase beta subunits is found [5] in some bacterial apparatus involved in a specialized protein export pathway that proceeds without signal peptide cleavage. This protein is known as fliI in Bacillus and Salmonella, Spa47 (mxiB) in Shigella flexneri, HrpB6 in Xanthomonas campestris and yscN in Yersinia virulence plasmids.To detect these ATPase subunits, a segment of ten amino-acid residues, containing two conserved serines, as a signature pattern was selected. The first serine seems to be important for catalysis - in the ATPase alpha chain at least - as its mutagenesis causes catalytic impairment.

Consensus pattern: P-[SAP]-[LIV]-[DNH]-x(3)-S-x-S [The first S is a putative active site residue]

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).

[2] Senior A.E. Physiol. Rev. 68:177-231(1988).

[3] Nelson N. J. Bioenerg. Biomembr. 21:553-571(1989).

[4] Gogarten J.P., Kibak H., Dittrich P., Taiz L., Bowman E.J., Bowman B.J., Manolson M.F., Poole R.J., Date T., Oshima T., Konishi J., Denda K., Yoshida M. Proc. Natl. Acad. Sci. U.S.A. 86:6661-665(1989).

[5] Dreyfus G., Williams A.W., Kawagishi I., MacNab R.M. J. Bacteriol. 175:3131-3138(1993).

15. (ATP synt ab C)

ATP synthase ab C terminal.

Number of members: 190

[1] Abrahams JP, Leslie AG, Lutter R, Walker JE; “Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria.” Nature 1994;370:621-628.

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16. (A deaminase)

Adenosine and AMP deaminase signature

Adenosine deaminase catalyzes the hydrolytic deamination ofadenosine into inosine. AMP deaminase catalyzes the hydrolytic deamination of AMP into IMP. It has been shown [1] that these two types of enzymes share three regions of sequence similarities; these regions are centered on residues which are proposed to play an important role in the catalytic mechanism of these two enzymes. One of these regions, containing two conserved aspartic acid residues that are potential active site residues was selected.

Consensus pattern: [SA]-[LIVM]-[NGS]-[STA]-D-D-P [The two D's are putative active site residues]

[1] Chang Z., Nygaard P., Chinault A.C., Kellems R.E. Biochemistry 30:2273-2280(1991).

17. (Acetyltransf)

Acetyltransferase (GNAT) family.

This family contains proteins with N-acetyltransferase functions.

[1] Neuwald AF, Landsman D; Trends Biochem Sci 1997;22:154-155.

18. (Aconitase C)

Aconitase family signature

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Aconitase (aconitate hydratase) (EC 4.2.1.3) [1] is the enzyme from the tricarboxylic acid cycle that catalyzes the reversible isomerization of citrate and isocitrate. Cis-aconitate is formed as an intermediary product during the course of the reaction. In eukaryotes two isozymes of aconitase are known to exist: one found in the mitochondrial matrix and the other found in the cytoplasm. Aconitase, in its active form, contains a 4Fe-4S iron-sulfur cluster; three cysteine residues have been shown to be ligands of the 4Fe-4S cluster.It has been shown that the aconitase family also contains the followingproteins: - Iron-responsive element binding protein (IRE-BP). IRE-BP is a cytosolic protein that binds to iron-responsive elements (IREs). IREs are stem-loop structures found in the 5'UTR of ferritin, and delta aminolevulinic acid synthase mRNAs, and in the 3'UTR of transferrin receptor mRNA. IRE-BP also express aconitase activity. - 3-isopropylmalate dehydratase (EC 4.2.1.33) (isopropylmalate isomerase), the enzyme that catalyzes the second step in the biosynthesis of leucine. - Homoaconitase (EC 4.2.1.36) (homoaconitate hydratase), an enzyme that participates in the alpha-aminoadipate pathway of lysine biosynthesis and that converts cis-homoaconitate into homoisocitric acid. - Esherichia coli protein ybhJ.As a signature for proteins from the aconitase family, two conserved regions that contain the three cysteine ligands of the 4Fe-4Scluster were selected.

Consensus pattern: [LIVM]-x(2)-[GSACIVM]-x-[LIV]-[GTIV]-[STP]-C-x(0,1)-T-N-[GSTANI]-x(4)-[LIVMA] [C binds the iron-sulfur center]

Consensus pattern: G-x(2)-[LIVWPQ]-x(3)-[GAC]-C-[GSTAM]-[LIMPTA]-C-[LIMV]-[GA] [The two C's bind the iron-sulfur center]

[1] Gruer M.J., Artymiuk P.J., Guest J.R. Trends Biochem. Sci. 22:3-6(1997).

19. (Acyl-CoA dh)

Acyl-CoA dehydrogenases signatures

Acyl-CoA dehydrogenases [1,2,3] are enzymes that catalyze the alpha, beta-dehydrogenation of acyl-CoA esters and transfer electrons to ETF, the electron transfer protein. Acyl-CoA dehydrogenases are FAD flavoproteins. This family currently includes: - Five eukaryotic isozymes that catalyze the first step of the beta-oxidation cycles for fatty acids with various chain lengths. These are short (SCAD) (EC 1.3.99.2), medium (MCAD) (EC 1.3.99.3), long (LCAD) (EC 1.3.99.13), very-long (VLCAD) and short/branched (SBCAD) chain acyl-CoA dehydrogenases. These enzymes are located in the mitochondrion. They are all homotetrameric proteins of about 400 amino acid residues except VLCAD which is a dimer and which contains, in its mature form, about 600 residues. - Glutaryl-CoA dehydrogenase (EC 1.3.99.7) (GCDH), which is involved in the catabolism of lysine, hydroxylysine and tryptophan. - Isovaleryl-CoA dehydrogenase (EC 1.3.99.10) (IVD), involved in the catabolism of leucine. - Acyl-coA dehydrogenases acsA and mmgC from Bacillus subtilis. - Butyryl-CoA dehydrogenase (EC 1.3.99.2) from Clostridium acetobutylicum. - Escherichia coli protein caiA [4]. - Escherichia coli protein aidB. Two conserved regions were selected as signature patterns. The first is located in the center of these enzymes, the second in the C-terminal section.

Consensus pattern: [GAC]-[LIVM]-[ST]-E-x(2)-[GSAN]-G-[ST]-D-x(2)-[GSA]

Consensus pattern: [QDE]-x(2)-G-[GS]-x-G-[LIVMFY]-x(2)-[DEN]-x(4)-[KR]-x(3)- [DEN]

[1] Tanaka K., Ikeda, Matsubara Y., Hyman D.B. Enzyme 38:91-107(1987).

[2] Matsubara Y., Indo Y., Naito E., Ozasa H., Glassberg R., Vockley J., Ikeda Y., Kraus J., Tanaka K. J. Biol. Chem. 264:16321-16331(1989).

[3] Aoyama T., Ueno I., Kamijo T., Hashimoto T. J. Biol. Chem. 269:19088-19094(1994).

[4] Eichler K., Bourgis F., Buchet A., Kleber H.-P., Mandrand-Berthelot M.-A. Mol. Microbiol. 13:775-786(1994).

20. (Acyl transf)

Acyl transferase domain

Number of members: 161

[1] Serre L, Verbree EC, Dauter Z, Stuitje AR, Derewenda ZS; Medline: 95286570 "The Escherichia coli malonyl-CoA:acyl carrier protein transacylase at 1.5-A resolution. Crystal structure of a fatty acid synthase component." J Biol Chem 1995;270:12961-12964.

09553261.061500

21. Acylphosphatase signatures

Acylphosphatase (EC 3.6.1.7) [1,2] catalyzes the hydrolysis of various acylphosphate carboxyl-phosphate bonds such as carbamyl phosphate, succinylphosphate, 1,3-diphosphoglycerate, etc. The physiological role of this enzymeis not yet clear. Acylphosphatase is a small protein of around 100 amino-acid residues. There are two known isozymes. One seems to be specific to muscular tissues, the other, called 'organ-common type', is found in many different tissues.While acylphosphatase have been so far only characterized in vertebrates,there are a number of bacterial and archebacterial hypothetical proteins that are highly similar to that enzyme and that probably possess the same activity.These proteins are: - Escherichia coli hypothetical protein yccX. - Bacillus subtilis hypothetical protein yflL. - Archaeoglobus fulgidus hypothetical protein AF0818. Two conserved regions were selected as signature patterns. The first is located in the N-terminal section, while the second is found in the central part ofthe protein sequence.

Consensus pattern: [LIV]-x-G-x-V-Q-G-V-x-[FM]-R

Consensus pattern: G-[FYW]-[AVC]-[KRQAM]-N-x(3)-G-x-V-x(5)-G

[1] Stefani M., Ramponi G. Life Chem. Rep. 12:271-301(1995).

[2] Stefani M., Taddei N., Ramponi G. Cell. Mol. Life Sci. 53:141-151(1997).

22. (Adap comp sub)

Clathrin adaptor complexes medium chain signatures.

Clathrin coated vesicles (CCV) mediate intracellular membrane traffic such asreceptor mediated endocytosis. In addition to clathrin, the CCV are composed of a number of other components including oligomeric complexes which are knownas adaptor or clathrin assembly proteins (AP) complexes [1]. The adaptor complexes are believed to interact with the cytoplasmic tails of membrane proteins, leading to their selection and concentration. In mammals two type of adaptor complexes are known: AP-1 which is associated with the Golgi complex and AP-2 which is associated with the plasma membrane. Both AP-1 and AP-2 are heterotetramers that consist of two large chains - the adaptins - (gamma and beta' in AP-1; alpha and beta in AP-2); a medium chain (AP47 in AP-1; AP50 inAP-2) and a small chain (AP19 in AP-1; AP17 in AP-2). The medium chains of AP-1 and AP-2 are evolutionary related proteins of about 50 Kd. Homologs of AP47 and AP50 have also been found in Caenorhabditis elegans (genes unc-101 and ap50) [2] and yeast (gene APM1 or YAP54) [3].Some more divergent, but clearly evolutionary related proteins have also been found in yeast: APM2 and YBR288c., Two conserved regions were selected as signature patterns, one located in the N-terminal region, the other from the central section of these proteins.

Consensus pattern: [IVT]-[GSP]-W-R-x(2,3)-[GAD]-x(2)-[HY]-x(2)-N-x- [LIVMAFY](3)-D-[LIVM]-[LIVMT]-E

Consensus pattern: [LIV]-x-F-I-P-P-x-G-x-[LIVMFY]-x-L-x(2)-Y

[1] Pearse B.M., Robinson M.S. Annu. Rev. Cell Biol. 6:151-171(1990).

[2] Lee J., Jongeward G.D., Sternberg P.W. Genes Dev. 8:60-73(1994).

[3] Nakayama Y., Goebl M., O'Brine G.B., Lemmon S., Pingchang C.E., Kirchhausen T. Eur. J. Biochem. 202:569-574(1991).

23. (Adenylsucc synt)
Adenylosuccinate synthetase signatures

Adenylosuccinate synthetase (EC 6.3.4.4) [1] plays an important role in purinebiosynthesis, by catalyzing the GTP-dependent conversion of IMP and aspartic acid to AMP. Adenylosuccinate synthetase has been characterized from various sources ranging from Escherichia coli (gene purA) to vertebrate tissues. Invertebrates, two isozymes are present - one involved in purine biosynthesis and the other in the purine nucleotide cycle. Two conserved regions were selected as signature patterns. The first one is a perfectly conserved octapeptide located in the N-terminal section and which is involved in GTP-binding [2]. The second one includes a lysine residue known [2] to be essential for the enzyme's activity.

Consensus pattern: Q-W-G-D-E-G-K-G

Consensus pattern: G-I-[GR]-P-x-Y-x(2)-K-x(2)-R [K is the active site residue]

[1] Wiesmueller L., Wittbrodt J., Noegel A.A., Schleicher M. J. Biol. Chem. 266:2480-2485(1991).
[2] Silva M.M., Poland B.W., Hoffman C.R., Fromm H.J., Honzatko R.B. J. Mol. Biol. 254:431-446(1995).
[3] Bouyoub A., Barbier G., Forterre P., Labedan B. 2.3.CO;2-"J. Mol. Biol. 261:144-154(1996).

24. (AdoHcyase)
S-adenosyl-L-homocysteine hydrolase signatures

S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) (AdoHcyase) is an enzyme of the activated methyl cycle, responsible for the reversible hydratation of S-adenosyl-L-homocysteine into adenosine and homocysteine. AdoHcyase is anubiquitous enzyme which binds and requires NAD+ as a cofactor. AdoHcyase is a highly conserved protein [1] of about

430 to 470 amino acids. Two highly conserved regions were selected as signature patterns. The first pattern is located in the N-terminal section; the second is derived from aglycine-rich region in the central part of AdoHcyase; a region thought to be involved in NAD-binding.

Consensus pattern: [GSA]-[CS]-N-x-[FYLM]-S-[ST]-[QA]-[DEN]-x-[AV]-[AT]-[AD]-[AC]-[LIVMCG]

Consensus pattern: [GA]-[KS]-x(3)-[LIV]-x-G-[FY]-G-x-[VC]-G-[KRL]-G-x-[ASC]

[1] Sganga M.W., Aksamit R.R., Cantoni G.L., Bauer C.E. Proc. Natl. Acad. Sci. U.S.A. 89:6328-6332(1992).

09555385.061501

25. AhpC/TSA family

This family contains proteins related to alkyl hydroperoxide reductaseComment: (AhpC) and thiol specific antioxidant (TSA).

[1] Chae HZ, Robison K, Poole LB, Church G, Storz G, Rhee SG, Proc Natl Acad Sci U S A 1994;91:7017-7021

26. (Aldose epim)

Aldose 1-epimerase putative active site Aldose 1-epimerase (EC 5.1.3.3) (mutarotase) is the enzyme responsible for the anomeric interconversion of D-glucose and other aldoses between their alpha- and beta-forms. The sequence of mutarotase from two bacteria, Acinetobacter calcoaceticus and Streptococcus thermophilus is available [1]. It has also been shown that, on the basis of extensive sequence similarities, a mutarotase domain seem to be present in the C-terminal half of the fungal GAL10 protein which encodes, in the N-terminal part, for UDP-glucose 4-epimerase. The best conserved region in the sequence of mutarotase is centered around a conserved histidine residue which may be involved in the catalytic mechanism.

Consensus pattern: [NS]-x-T-N-H-x-Y-[FW]-N-[LI]

[1] Poolman B., Royer T.J., Mainzer S.E., Schmidt B.F. J. Bacteriol. 172:4037-4047(1990).

27. (AlkA DNA repair)

Alkylbase DNA glycosidases alkA family signature

Alkylbase DNA glycosidases [1] are DNA repair enzymes that hydrolyzes the deoxyribose N-glycosidic bond to excise various alkylated bases from a damaged DNA polymer. In Escherichia coli there are two alkylbase DNA glycosidases: one (gene tag)which is constitutively expressed and which is specific for the removal of 3-methyladenine (EC 3.2.2.20), and one (gene alkA) which is induced during adaptation to alkylation and which can remove a variety of alkylation products (EC 3.2.2.21). Tag and alkA do not share any region of sequence similarity. In yeast there is an alkylbase DNA glycosidase (gene MAG1) [2,3], which can remove 3-methyladenine or 7-methyladenine and which is structurally related to alkA. MAG and alkA are both proteins of about 300 amino acid residues. While the C- and N-terminal ends appear to be unrelated, there is a central region of about 130 residues which is well conserved. A portion of this region has been selected as a signature pattern .

Consensus pattern: G-I-G-x-W-[ST]-[AV]-x-[LIVMFY](2)-x-[LIVM]-x(8)-[MF]-x(2)-[ED]-D

[1] Lindahl T., Sedgwick B. Annu. Rev. Biochem. 57:133-157(1988).
[2] Berdal K.G., Bjoras M., Bjelland S., Seeberg E.C. EMBO J. 9:4563-4568(1990).
[3] Chen J., Derfler B., Samson L. EMBO J. 9:4569-4575(1990).

28. Ammonium transporters signature

A number of proteins involved in the transport of ammonium ions across amembrane as well as some yet uncharacterized proteins have been shown [1,2] to be evolutionary related. These proteins are: - Yeast ammonium transporters MEP1, MEP2 and MEP3. - Arabidopsis thaliana high affinity ammonium transporter (gene AMT1). - Corynebacterium glutamicum ammonium and methylammonium transport system. - Escherichia coli putative ammonium transporter amtB. - Bacillus subtilis nrgA. - Mycobacterium tuberculosis hypothetical protein MtCY338.09c. - Synechocystis strain PCC 6803 hypothetical proteins sll0108, sll0537 and sll1017. - Methanococcus jannaschii hypothetical proteins MJ0058 and MJ1343. - Caenorhabditis elegans hypothetical proteins C05E11.4, F49E11.3 and M195.3. As expected by their transport function, these proteins are highly hydrophobic and seem to contain from 10 to 12 transmembrane domains. The best conserved region seems to be located in the fifth (or sixth) transmembrane region and is used as a signature pattern.

Consensus pattern: D-[FYWS]-A-G-[GSC]-x(2)-[IV]-x(3)-[SAG](2)-x(2)-[SAG]- [LIVMF]-x(3)-[LIVMFYWA](2)-x-[GK]-x-R

[1] Ninnemann O., Janniaux J.-C., Frommer W.B. EMBO J. 13:3464-3471(1994).
[2] Siewe R.M., Weil B., Burkovski A., Eikmanns B.J., Eikmanns M., Kraemer R. J. Biol. Chem. 271:5398-5403(1996).
[3] Saier M.H. Jr. Adv. Microbiol. Physiol. 40:81-136(1998).

29. (Arch_histone)
CBF/NF-Y subunits signatures

Diverse DNA binding proteins are known to bind the CCAAT box, a common cis-acting element found in the promoter and enhancer regions of a large number of genes in eukaryotes. Amongst these proteins is one known as the CCAAT-binding factor (CBF) or NF-Y [1]. CBF is a heteromeric transcription factor that consists of two different components both needed for DNA-binding. The HAP protein complex of yeast binds to the upstream activation site of cytochrome C iso-1 gene (CYC1) as well as other genes involved in mitochondrial electron transport and activates their expression. It also recognizes the sequence CCAAT and is structurally and evolutionary related to CBF. The first subunit of CBF, known as CBF-A or NF-YB in vertebrates, HAP3 in budding yeast and as php3 in fission yeast, is a protein of 116 to 210 amino-acid residues which contains a highly conserved central domain of about 90residues. This domain seems to be involved in DNA-binding; a signature pattern had been developed from its central part. The second subunit of CBF, known as CBF-B or NF-YA in vertebrates, HAP2 in budding yeast and php2 in fission yeast, is a protein of 265 to 350 amino-acid residues which contains a highly conserved region of about 60 residues. This region, called the 'essential core' [2], seems to consist of two subdomains: an N-terminal subunit-association domain and a C-terminal DNA recognition domain. A signature pattern has been developed from a section of the subunit-association domain.

Consensus pattern: C-V-S-E-x-I-S-F-[LIVM]-T-[SG]-E-A-[SC]-[DE]-[KRQ]-C-

Consensus pattern: Y-V-N-A-K-Q-Y-x-R-I-L-K-R-R-x-A-R-A-K-L-E-

[1] Li X.-Y., Mantovani R., Hooft van Huijsduijnen R., Andre I., Benoist C., Mathis D. Nucleic Acids Res. 20:1087-1091(1992).

[2] Olesen J.T., Fikes J.D., Guarente L. Mol. Cell. Biol. 11:611-619(1991).

30. Argininosuccinate synthase signatures

Argininosuccinate synthase (EC 6.3.4.5) (AS) is a urea cycle enzyme that catalyzes the penultimate step in arginine biosynthesis: the ATP-dependent ligation of citrulline to aspartate to form argininosuccinate, AMP andpyrophosphate [1,2].In humans, a defect in the AS gene causes citrullinemia, a genetic disease characterized by severe vomiting spells and mental retardation.AS is a homotetrameric enzyme of chains of about 400 amino-acid residues. Anarginine seems to be important for the enzyme's catalytic mechanism. The sequences of AS from various prokaryotes, archaebacteria and eukaryotes show significant similarity. Two signature patterns have been selected for AS. The first is a highly conserved stretch of nine residues located in the N-terminal extremity of these enzymes, the second is derived from a conserved region which contains one of the conserved arginine residues.

Consensus pattern: [AS]-[FY]-S-G-G-[LV]-D-T-[ST]-

Consensus pattern: G-x-T-x-K-G-N-D-x(2)-R-F-

[1] van Vliet F., Crabeel M., Boyen A., Tricot C., Stalon V., Falmagne P., Nakamura Y., Baumberg S., Glansdorff N. Gene 95:99-104(1990).
[2] Morris C.J., Reeve J.N. J. Bacteriol. 170:3125-3130(1988).

31. Armadillo/beta-catenin-like repeats

Approx. 40 amino acid repeat. Tandem repeats form super-helix of helices that is proposed to mediate interaction of beta-catenin with its ligands. CAUTION: This family does not contain all known armadillo repeats.

[1] Huber AH, Nelson WJ, Weis WI, Cell 1997;90:871-882.
[2] Gumbiner BM, Curr Opin Cell Biol 1995;7:634-640.
[3] Cavallo R, Rubenstein D, Peifer M, Curr Opin Genet Dev 1997;7:459-466.
[4] Su LK, Vogelstein B, Kinzler KW, Science 1993;262:1734-1737.
[5] Masiarz FR, Munemitsu S, Polakis P Science 1993;262:1731-1734
[6] Peifer M, Wieschaus E, Cell 1990;63:1167-1176.

32. (Asn Synthase)

Asparagine synthase

This family is always found associated with GATase_2. Members of this family catalyse the conversion of aspartate to asparagine.

33. Asparaginase_2

Asparaginase 12 members

34. (Aspartyl tRNA N)

Aminoacyl-transfer RNA synthetases class-II signatures

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7]. Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns have been derived from two of these regions.

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Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]
Consensus pattern: [GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).
[2] Delarue M., Moras D. BioEssays 15:675-687(1993).
[3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).
[4] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).
[5] Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).
[6] Cusack S. Biochimie 75:1077-1081(1993).
[7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990).
[8] Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

35. (ArfGap) Putative GTP-ase activating protein for Arf. Putative zinc fingers with GTPase activating proteins (GAPs) towards the small GTPase, Arf. The GAP of ARD1 stimulates

GTPase hydrolysis for ARD1 but not ARFs. Number of members: 34

[1]Medline: 96324970. Identification and cloning of centaurin-alpha. A novel phosphatidylinositol 3,4,5-trisphosphate-binding protein from rat brain. Hammonds-Odie LP, Jackson TR, Profit AA, Blader IJ, Turck CW, Prestwich GD, Theibert AB; J Biol Chem 1996;271:18859-18868.

[2]Medline: 97296423. A target of phosphatidylinositol 3,4,5-trisphosphate with a zinc finger motif similar to that of the ADP-ribosylation -factor GTPase-activating protein and two pleckstrin homology domains. Tanaka K, Imajoh-Ohmi S, Sawada T, Shirai R, Hashimoto Y, Iwasaki S, Kaibuchi K, Kanaho Y, Shirai T, Terada Y, Kimura K, Nagata S, Fukui Y; Eur J Biochem 1997;245:512-519.

[3] 98112795. Molecular characterization of the GTPase-activating domain of ADP-ribosylation factor domain protein 1 (ARD1). Vitale N, Moss J, Vaughan M; J Biol Chem 1998;273:2553-2560.

09565261.061702

36. Apolipoprotein. Apolipoprotein A1/A4/E family. This family includes: Swiss:P02647 Apolipoprotein A-I. Swiss:P06727 Apolipoprotein A-IV. Swiss:P02649 Apolipoprotein E. These proteins contain several 22 residue repeats which form a pair of alpha helices. Number of members: 42

[1]Medline: 91289138. Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. Wilson C, Wardell MR, Weisgraber KH, Mahley RW, Agard DA; Science 1991;252:1817-1822.

37. Amino acid permeases signature

Amino acid permeases are integral membrane proteins involved in the transport of amino acids into the cell. A number of such proteins have been found to be evolutionary related [1,2,3]. These proteins are: - Yeast general amino acid permeases (genes GAP1, AGP2 and AGP3). - Yeast basic amino acid permease (gene ALP1). - Yeast Leu/Val/Ile permease (gene BAP2). - Yeast arginine permease (gene CAN1). - Yeast dicarboxylic amino acid permease (gene DIP5). - Yeast asparagine/glutamine permease (gene AGP1). - Yeast glutamine permease (gene GNP1). - Yeast histidine permease (gene HIP1). - Yeast lysine permease (gene LYP1). - Yeast proline permease (gene PUT4). - Yeast valine and tyrosine permease (gene VAL1/TAT1). - Yeast tryptophan permease (gene TAT2/SCM2). - Yeast choline transport protein (gene HNM1/CTR1). - Yeast GABA permease (gene UGA4). - Yeast hypothetical protein YKL174c. - Fission yeast protein isp5. - Fission yeast hypothetical protein SpAC8A4.11 - Fission yeast hypothetical protein SpAC11D3.08c. - Emericella nidulans proline transport protein (gene prnB). - Trichoderma harzianum amino acid permease INDA1. - Salmonella typhimurium L-asparagine permease (gene ansP). - Escherichia coli aromatic amino acid transport protein (gene aroP). - Escherichia coli D-serine/D-alanine/glycine transporter (gene cycA). - Escherichia coli GABA permease (gene gabP). - Escherichia coli lysine-specific permease (gene lysP). - Escherichia coli phenylalanine-specific permease (gene pheP). - Salmonella typhimurium proline-specific permease (gene proY). - Escherichia coli and Klebsiella pneumoniae hypothetical protein yeeF. - Escherichia coli and Salmonella typhimurium hypothetical protein yifK. - Bacillus subtilis permeases rocC and rocE which probably transports arginine or ornithine. These proteins seem to contain up to 12 transmembrane segments. As a signature for this family of proteins, the best conserved region which is located in the second transmembrane segment has been selected.

0959560 .051502

Consensus pattern: [STAGC]-G-[PAG]-x(2,3)-[LIVMFYWA](2)-x-[LIVMFYW]-x-[LIVMFWSTAGC](2)-[STAGC]-x(3)-[LIVMFYWT]-x-[LIVMST]-x(3)- [LIVMCTA]-[GA]-E-x(5)-[PSAL]-

[1] Weber E., Chevalier M.R., Jund R. J. Mol. Evol. 27:341-350(1988).
[2] Vandenbol M., Jauniaux J.-C., Grenson M. Gene 83:153-159(1989).
[3] Reizer J., Finley K., Kakuda D., McLeod C.L., Reizer A., Saier M.H. Jr. Protein Sci. 2:20-30(1993).

38. aakinase (1) Glutamate 5-kinase signature

Glutamate 5-kinase (EC 2.7.2.11) (gamma-glutamyl kinase) (GK) is the enzyme that catalyzes the first step in the biosynthesis of proline from glutamate, the ATP-dependent phosphorylation of L-glutamate into L-glutamate 5-phosphate. In eubacteria (gene proB) and yeast [1] (gene PRO1), GK is a monofunctional protein, while in plants and mammals, it is a bifunctional enzyme (P5CS) [2]that consists of two domains: a N-terminal GK domain and a C-terminal gamma-glutamyl phosphate reductase domain (EC 1.2.1.41) (see <PDOC00940>).As a signature pattern, a highly conserved glycine-and alanine-rich region located in the central section of these enzymes has been selected. Yeast hypothetical protein YHR033w is highly similar to GK.

Consensus pattern: [GSTN]-x(2)-G-x-G-[GC]-[IM]-x-[STA]-K-[LIVM]-x-[SA]-[TCA]-x(2)-[GALV]-x(3)-G-

[1] Li W., Brandriss M.C. J. Bacteriol. 174:4148-4156(1992).
[2] Hu C.-A.A., Delauney A.J., Verma D.P.S. Proc. Natl. Acad. Sci. U.S.A. 89:9354-9358(1992).

09655962.050700

aakinase (2) Aspartokinase signature
Aspartokinase (EC 2.7.2.4) (AK) [1] catalyzes the phosphorylation of aspartate. The product of this reaction can then be used in the biosynthesis of lysine or in the pathway leading to homoserine, which participates in the biosynthesis of threonine, isoleucine and methionine. In Escherichia coli, there are three different isozymes which differ in their sensitivity to repression and inhibition by Lys, Met and Thr. AK1 (gene thrA) and AK2 (gene metL) are bifunctional enzymes which both consist of an N- terminal AK domain and a C-terminal homoserine dehydrogenase domain. AK1 is involved in threonine biosynthesis and AK2, in that of methionine. The third isozyme, AK3 (gene lysC), is monofunctional and involved in lysine synthesis. In yeast, there is a single isozyme of AK (gene HOM3). As a signature pattern for AK, a conserved region located in the N-terminal extremity has been selected.

Consensus pattern: [LIVM]-x-K-[FY]-G-G-[ST]-[SC]-[LIVM]-

[1] Rafalski J.A., Falco S.C. J. Biol. Chem. 263:2146-2151(1988).

aakinase (3) Gamma-glutamyl phosphate reductase signature
Gamma-glutamyl phosphate reductase (EC 1.2.1.41) (GPR) is the enzyme that catalyzes the second step in the biosynthesis of proline from glutamate, the NADP-dependent reduction of

L-glutamate 5-phosphate into L-glutamate 5-semialdehyde and phosphate. In eubacteria (gene proA) and yeast [1] (gene PRO2), GPR is a monofunctional protein, while in plants and mammals, it is a bifunctional enzyme (P5CS) [2]that consists of two domains: a N-terminal glutamate 5-kinase domain(EC 2.7.2.11) (see <PDOC00701>) and a C-terminal GPR domain. As a signature pattern, a conserved region that contains two histidine residues has been selected. This region is located in the last third of GPR.

Consensus pattern: V-x(5)-A-[LIV]-x-H-I-x(2)-[HY]-[GS]-[ST]-x-H-[ST]-[DE]-x- I-

[1] Pearson B.M., Hernando Y., Payne J., Wolf S.S., Kalogeropoulos A., Schweizer M. Yeast 12:1021-1031(1996).

[2] Hu C.-A.A., Delauney A.J., Verma D.P.S. Proc. Natl. Acad. Sci. U.S.A. 89:9354-9358(1992).

09555320 . 061901

39. (abhydrolase) alpha/beta hydrolase fold. This catalytic domain is found in a very wide range of enzymes.

[1] Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM, Harel M, Remington SJ, Silman I, Schrag J, Sussman JL, Verschueren KHG, Goldman A, Protein Eng 1992;5:197-211.

40. (Acid phosphat) Histidine acid phosphatases signatures

Acid phosphatases (EC 3.1.3.2) are a heterogeneous group of proteins that hydrolyze phosphate esters, optimally at low pH. It has been shown [1] that a number of acid phosphatases, from both prokaryotes and eukaryotes, share two regions of sequence similarity, each centered around a conserved histidine residue. These two histidines seem to be involved in the enzymes' catalytic mechanism [2,3]. The first histidine is located in the N-terminal section and forms a phosphohistidine intermediate while the second is located in the C- terminal section and possibly acts as proton donor. Enzymes belonging to this family are called 'histidine acid phosphatases' and are listed below:

- Escherichia coli pH 2.5 acid phosphatase (gene appA).

□

- Escherichia coli glucose-1-phosphatase (EC 3.1.3.10) (gene agp).
- Yeast constitutive and repressible acid phosphatases (genes PHO3 and PHO5).
- Fission yeast acid phosphatase (gene pho1).
- Aspergillus phytases A and B (EC 3.1.3.8) (gene phyA and phyB).
- Mammalian lysosomal acid phosphatase.
- Mammalian prostatic acid phosphatase.
- Caenorhabditis elegans hypothetical proteins B0361.7, C05C10.1, C05C10.4 and F26C11.1.

Consensus pattern[LIVM]-x(2)-[LIVMA]-x(2)-[LIVM]-x-R-H-[GN]-x-R-x-[PAS] [H is the phosphohistidine residue]

□

Consensus pattern[LIVMF]-x-[LIVMFAG]-x(2)-[STAGI]-H-D-[STANQ]-x-[LIVM]-x(2)-[LIVMFY]-x(2)-[STA] [H is an active site residue] Sequences known to belong to this class detected by the patternALL, except for rat prostatic acid phosphatase which seems to have Tyr instead of the active site His

[1] van Etten R.L., Davidson R., Stevis P.E., MacArthur H., Moore D.L. J. Biol. Chem. 266:2313-2319(1991).
[2] Ostanin K., Harms E.H., Stevis P.E., Kuciel R., Zhou M.-M., van Etten R.L. J. Biol. Chem. 267:22830-22836(1992).
[3] Schneider G., Lindqvist Y., Vihko P. EMBO J. 12:2609-2615(1993).

41. Aconitase family signatures

Aconitase (aconitate hydratase) (EC 4.2.1.3) [1] is the enzyme from the tricarboxylic acid cycle that catalyzes the reversible isomerization of citrate and isocitrate. Cis-aconitate is formed as an intermediary product during the course of the reaction. In eukaryotes two isozymes of aconitase are known to exist: one found in the mitochondrial matrix and the other found in the cytoplasm. Aconitase, in its active form, contains a 4Fe-4S iron-sulfur cluster; three cysteine residues have been shown to be ligands of the 4Fe-4S cluster. It has been shown that the aconitase family also contains the following proteins: - Iron-responsive element binding protein (IRE-BP). IRE-BP is a cytosolic protein that binds to iron-responsive elements (IREs). IREs are stem-loop structures found in the 5'UTR of ferritin, and delta aminolevulinic acid synthase mRNAs, and in the 3'UTR of transferrin receptor mRNA. IRE-BP also express aconitase activity. - 3-isopropylmalate dehydratase (EC 4.2.1.33) (isopropylmalate isomerase), the enzyme that catalyzes the second step in the biosynthesis of leucine. - Homoaconitase (EC 4.2.1.36) (homoaconitate hydratase), an enzyme that participates in the alpha-aminoadipate pathway of lysine biosynthesis and that converts cis-homoaconitate into homoisocitric acid. - Esherichia coli protein ybhJ

Consensus pattern: [LIVM]-x(2)-[GSACIVM]-x-[LIV]-[GTIV]-[STP]-C-x(0,1)-T-N-[GSTANI]-x(4)-[LIVMA] [C binds the iron-sulfur center]
Consensus pattern: G-x(2)-[LIVWPQ]-x(3)-[GAC]-C-[GSTAM]-[LIMPTA]-C-[LIMV]-[GA] [The two C's bind the iron-sulfur center]-

[1] Gruer M.J., Artymiuk P.J., Guest J.R. Trends Biochem. Sci. 22:3-6(1997).

09555356.051701

42. Actins signatures

Actins [1 to 4] are highly conserved contractile proteins that are present in all eukaryotic cells. In vertebrates there are three groups of actin isoforms: alpha, beta and gamma. The alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus. The beta and gamma actins co-exists in most cell types as components of the cytoskeleton and as mediators of internal cell motility. In plants [5] there are many isoforms which are probably involved in a variety of functions such as cytoplasmic streaming, cell shape determination, tip growth, graviperception, cell wall deposition, etc. Actin exists either in a monomeric form (G-actin) or in a polymerized form (F-actin). Each actin monomer can bind a molecule of ATP; when polymerization occurs, the ATP is hydrolyzed. Actin is a protein of from 374 to 379 amino acid residues. The structure of actin has been highly conserved in the course of evolution. Recently some divergent actin-like proteins have been identified in several species. These proteins are: - Centractin (actin-RPV) from mammals, fungi (yeast ACT5, Neurospora crassa ro-4) and Pneumocystis carinii (actin-II). Centractin seems to be a component of a multi-subunit centrosomal complex involved in microtubule based vesicle motility. This subfamily is also known as ARP1. - ARP2 subfamily which includes chicken ACTL, yeast ACT2, Drosophila 14D, C.elegans actC. - ARP3 subfamily which includes actin 2 from mammals, Drosophila 66B, yeast ACT4 and fission yeast act2. - ARP4 subfamily which includes yeast ACT3 and Drosophila 13E. Three signature patterns have been developed. The first two are specific to actins and span positions 54 to 64 and 357 to 365. The last signature picks up both actins and the actin-like proteins and corresponds to positions 106 to 118 in actins.

Consensus pattern: [FY]-[LIV]-G-[DE]-E-A-Q-x-[RKQ](2)-G-
Consensus pattern: W-[IV]-[STA]-[RK]-x-[DE]-Y-[DNE]-[DE]-
Consensus pattern: [LM]-[LIVM]-T-E-[GAPQ]-x-[LIVMFYWHQ]-N-[PSTAQ]-x(2)-N-[KR]-

[1] Sheterline P., Clayton J., Sparrow J.C. (In) Actins, 3rd Edition, Academic Press Ltd, London, (1996).
[2] Pollard T.D., Cooper J.A. Annu. Rev. Biochem. 55:987-1036(1986).
[3] Pollard T.D. Curr. Opin. Cell Biol. 1:33-40(1990).
[4] Rubenstein P.A. BioEssays 12:309-315(1990).
[5] Meagher R.B., McLean B.G. Cell Motil. Cytoskeleton 16:164-166(1990).

43. Adenylate kinase signature

Adenylate kinase (EC 2.7.4.3) (AK) [1] is a small monomeric enzyme that catalyzes the reversible transfer of MgATP to AMP (MgATP + AMP = MgADP + ADP).In mammals there are three different isozymes: - AK1 (or myokinase), which is cytosolic. - AK2, which is located in the outer compartment of mitochondria. - AK3 (or GTP:AMP phosphotransferase), which is located in the mitochondrial matrix and which uses MgGTP instead of MgATP.The sequence of AK has also been obtained from different bacterial species and from plants and fungi. Two other enzymes have been found to be evolutionary related to AK. These are: - Yeast uridylate kinase (EC 2.7.4.-) (UK) (gene URA6) [2] which catalyzes the transfer of a phosphate group from ATP to UMP to form UDP and ADP. - Slime mold UMP-CMP kinase (EC 2.7.4.14) [3] which catalyzes the transfer of a phosphate group from ATP to either CMP or UMP to form CDP or UDP and ADP. Several regions of AK family enzymes are well

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conserved, including the ATP-binding domains. The most conserved of all regions have been selected as a signature for this type of enzyme. This region includes an aspartic acid residue that is part of the catalytic cleft of the enzyme and that is involved in a salt bridge. It also includes an arginine residue whose modification leads to inactivation of the enzyme

Consensus pattern: [LIVMFYW](3)-D-G-[FYI]-P-R-x(3)-[NQ]-

[1] Schulz G.E. Cold Spring Harbor Symp. Quant. Biol. 52:429-439(1987).
[2] Liljelund P., Sanni A., Friesen J.D., Lacroute F. Biochem. Biophys. Res. Commun. 165:464-473(1989).
[3] Wiesmueller L., Noegel A.A., Barzu O., Gerisch G., Schleicher M. J. Biol. Chem. 265:6339-6345(1990).
[4] Kath T.H., Schmid R., Schaefer G. Arch. Biochem. Biophys. 307:405-410(1993).

44. (adh_short) Short-chain dehydrogenases/reductases family signature. The short-chain dehydrogenases/reductases family (SDR) [1] is a very large family of enzymes, most of which are known to be NAD- or NADP-dependent oxidoreductases. As the first member of this family to be characterized was Drosophila alcohol dehydrogenase, this family used to be called [2,3,4]'insect-type', or 'short-chain' alcohol dehydrogenases. Most member of this family are proteins of about 250 to 300 amino acid residues. The proteins currently known to belong to this family are listed below. - Alcohol dehydrogenase (EC 1.1.1.1) from insects such as Drosophila. - Acetoin dehydrogenase (EC 1.1.1.5) from Klebsiella terrigena (gene budC). - D-beta-hydroxybutyrate dehydrogenase (BDH) (EC 1.1.1.30) from mammals. - Acetoacetyl-CoA reductase (EC 1.1.1.36) from various bacterial species (gene phbB or phaB). - Glucose 1-dehydrogenase (EC 1.1.1.47) from Bacillus. - 3-beta-hydroxysteroid dehydrogenase (EC 1.1.1.51) from Comomonas testosteroni. - 20-beta-hydroxysteroid dehydrogenase (EC 1.1.1.53) from Streptomyces hydrogenans. - Ribitol dehydrogenase (EC 1.1.1.56) (RDH) from Klebsiella aerogenes. - Estradiol 17-beta-dehydrogenase (EC 1.1.1.62) from human. - Gluconate 5-dehydrogenase (EC 1.1.1.69) from Gluconobacter oxydans (gene gno). - 3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100) from Escherichia coli (gene fabG) and from plants. - Retinol dehydrogenase (EC 1.1.1.105) from mammals. - 2-deoxy-d-gluconate 3-dehydrogenase (EC 1.1.1.125) from Escherichia coli and Erwinia chrysanthemi

(gene kduD). - Sorbitol-6-phosphate 2-dehydrogenase (EC 1.1.1.140) from Escherichia coli (gene gutD) and from Klebsiella pneumoniae (gene sorD). - 15-hydroxyprostaglandin dehydrogenase (NAD+) (EC 1.1.1.141) from human. - Corticosteroid 11-beta-dehydrogenase (EC 1.1.1.146) (11-DH) from mammals. - 7-alpha-hydroxysteroid dehydrogenase (EC 1.1.1.159) from Escherichia coli (gene hdhA), Eubacterium strain VPI 12708 (gene baiA) and from Clostridium sordellii. - NADPH-dependent carbonyl reductase (EC 1.1.1.184) from mammals. - Tropinone reductase-I (EC 1.1.1.206) and -II (EC 1.1.1.236) from plants. - N-acylmannosamine 1-dehydrogenase (EC 1.1.1.233) from Flavobacterium strain 141-8. - D-arabinitol 2-dehydrogenase (ribulose forming) (EC 1.1.1.250) from fungi. - Tetrahydroxynaphthalene reductase (EC 1.1.1.252) from Magnaporthe grisea. - Pteridine reductase 1 (EC 1.1.1.253) (gene PTR1) from Leishmania. - 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase (EC 1.1.-.-) from Pseudomonas paucimobilis. - Cis-1,2-dihydroxy-3,4-cyclohexadiene-1-carboxylate dehydrogenase (EC 1.3.1. -) from Acinetobacter calcoaceticus (gene benD) and Pseudomonas putida (gene xylL). - Biphenyl-2,3-dihydro-2,3-diol dehydrogenase (EC 1.3.1.-) (gene bphB) from various Pseudomonaceae. - Cis-toluene dihydrodiol dehydrogenase (EC 1.3.1.-) from Pseudomonas putida (gene todD). - Cis-benzene glycol dehydrogenase (EC 1.3.1.19) from Pseudomonas putida (gene bnzE). - 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) from Escherichia coli (gene entA) and Bacillus subtilis (gene dhbA). - Dihydropteridine reductase (EC 1.6.99.7) (HDHPR) from mammals. - Lignin degradation enzyme ligD from Pseudomonas paucimobilis. - Agropine synthesis reductase from Agrobacterium plasmids (gene mas1). - Versicolorin reductase from Aspergillus parasiticus (gene VER1). - Putative keto-acyl reductases from Streptomyces polyketide biosynthesis operons. - A trifunctional hydratase-dehydrogenase-epimerase from the peroxisomal beta-oxidation system of Candida tropicalis. This protein contains two tandemly repeated 'short-chain dehydrogenase-type' domain in its N-terminal extremity. - Nodulation protein nodG from species of Azospirillum and Rhizobium which is probably involved in the modification of the nodulation Nod factor fatty acyl chain. - Nitrogen fixation protein fixR from Bradyrhizobium japonicum. - Bacillus subtilis protein dltE which is involved in the biosynthesis of D- alanyl-lipoteichoic acid. - Human follicular variant translocation protein 1 (FVT1). - Mouse adipocyte protein p27. - Mouse protein Ke 6. - Maize sex determination protein TASSELSEED 2. - Sarcophaga peregrina 25 Kd development specific protein. - Drosophila fat body protein P6. - A Listeria monocytogenes hypothetical protein encoded in the internalins gene region. - Escherichia coli hypothetical protein yciK. - Escherichia coli hypothetical protein ydfG. - Escherichia coli hypothetical protein yjgI. - Escherichia coli hypothetical protein yjgU. - Escherichia coli hypothetical protein yohF. - Bacillus subtilis hypothetical protein yoxD. - Bacillus subtilis hypothetical protein ywfD. - Bacillus subtilis hypothetical protein ywfH. - Yeast hypothetical protein YIL124w. - Yeast hypothetical protein YIR035c. - Yeast hypothetical protein YIR036c. - Yeast hypothetical protein YKL055c. - Fission yeast hypothetical protein SpAC23D3.11. One of the best conserved regions which includes two perfectly conserved residues, a tyrosine and a lysine has been selected as a signature pattern for this family of proteins. The tyrosine residue participates in the catalytic mechanism.

Consensus pattern: [LIVSPADNK]-x(12)-Y-[PSTAGNCV]-[STAGNQCIVM]-[STAGC]-K-{PC}-[SAGFYR]-[LIVMSTAGD]-x(2)-[LIVMFYW]-x(3)- [LIVMFYWGAPTHQ]-[GSACQRHM] [Y is an active site residue] -

[1] Joernvall H., Persson B., Krook M., Atrian S., Gonzalez-Duarte R., Jeffery J., Ghosh D. Biochemistry 34:6003-6013(1995).

[2] Villarroya A., Juan E., Egestad B., Joernvall H. Eur. J. Biochem. 180:191-197(1989).

[3] Persson B., Krook M., Joernvall H. Eur. J. Biochem. 200:537-543(1991).

[4] Neidle E.L., Hartnett C., Ornston N.L., Bairoch A., Rekik M., Harayama S. Eur. J. Biochem. 204:113-120(1992).

45. (adh_short_C2) Short-chain dehydrogenases/reductases family signature

The short-chain dehydrogenases/reductases family (SDR) [1] is a very large family of enzymes, most of which are known to be NAD- or NADP-dependent oxidoreductases. As the first member of this family to be characterized was Drosophila alcohol dehydrogenase, this family used to be called [2,3,4]'insect-type', or 'short-chain' alcohol dehydrogenases. Most member of this family are proteins of about 250 to 300 amino acid residues. The proteins currently known to belong to this family are listed below. - Alcohol dehydrogenase (EC 1.1.1.1) from insects such as Drosophila. - Acetoin dehydrogenase (EC 1.1.1.5) from Klebsiella terrigena (gene budC). - D-beta-hydroxybutyrate dehydrogenase (BDH) (EC 1.1.1.30) from mammals. - Acetoacetyl-CoA reductase (EC 1.1.1.36) from various bacterial species (gene phbB or phaB). - Glucose 1-dehydrogenase (EC 1.1.1.47) from Bacillus. - 3-

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beta-hydroxysteroid dehydrogenase (EC 1.1.1.51) from Comomonas testosteroni. - 20-beta-hydroxysteroid dehydrogenase (EC 1.1.1.53) from Streptomyces hydrogenans. - Ribitol dehydrogenase (EC 1.1.1.56) (RDH) from Klebsiella aerogenes. - Estradiol 17-beta-dehydrogenase (EC 1.1.1.62) from human. - Gluconate 5-dehydrogenase (EC 1.1.1.69) from Gluconobacter oxydans (gene gno). - 3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100) from Escherichia coli (gene fabG) and from plants. - Retinol dehydrogenase (EC 1.1.1.105) from mammals. - 2-deoxy-d-gluconate 3-dehydrogenase (EC 1.1.1.125) from Escherichia coli and Erwinia chrysanthemi (gene kduD). - Sorbitol-6-phosphate 2-dehydrogenase (EC 1.1.1.140) from Escherichia coli (gene gutD) and from Klebsiella pneumoniae (gene sorD). - 15-hydroxyprostaglandin dehydrogenase (NAD+) (EC 1.1.1.141) from human. - Corticosteroid 11-beta-dehydrogenase (EC 1.1.1.146) (11-DH) from mammals. - 7-alpha-hydroxysteroid dehydrogenase (EC 1.1.1.159) from Escherichia coli (gene hdhA), Eubacterium strain VPI 12708 (gene baiA) and from Clostridium sordellii. - NADPH-dependent carbonyl reductase (EC 1.1.1.184) from mammals. - Tropinone reductase-I (EC 1.1.1.206) and -II (EC 1.1.1.236) from plants. - N-acylmannosamine 1-dehydrogenase (EC 1.1.1.233) from Flavobacterium strain 141-8. - D-arabinitol 2-dehydrogenase (ribulose forming) (EC 1.1.1.250) from fungi. - Tetrahydroxynaphthalene reductase (EC 1.1.1.252) from Magnaporthe grisea. - Pteridine reductase 1 (EC 1.1.1.253) (gene PTR1) from Leishmania. - 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase (EC 1.1.-.-) from Pseudomonas paucimobilis. - Cis-1,2-dihydroxy-3,4-cyclohexadiene-1-carboxylate dehydrogenase (EC 1.3.1. -) from Acinetobacter calcoaceticus (gene benD) and Pseudomonas putida (gene xylL). - Biphenyl-2,3-dihydro-2,3-diol dehydrogenase (EC 1.3.1.-) (gene bphB) from various Pseudomonaceae. - Cis-toluene dihydrodiol dehydrogenase (EC 1.3.1.-) from Pseudomonas putida (gene todD). - Cis-benzene glycol dehydrogenase (EC 1.3.1.19) from Pseudomonas putida (gene bnzE). - 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase (EC 1.3.1.28) from Escherichia coli (gene entA) and Bacillus subtilis (gene dhbA). - Dihydropteridine reductase (EC 1.6.99.7) (HDHPR) from mammals. - Lignin degradation enzyme ligD from Pseudomonas paucimobilis. - Agropine synthesis reductase from Agrobacterium plasmids (gene mas1). - Versicolorin reductase from Aspergillus parasiticus (gene VER1). - Putative keto-acyl reductases from Streptomyces polyketide biosynthesis operons. - A trifunctional hydratase-dehydrogenase-epimerase from the peroxisomal beta-oxidation system of Candida tropicalis. This protein contains two tandemly repeated 'short-chain dehydrogenase-type' domain in its N-terminal extremity. - Nodulation protein nodG from species of Azospirillum and Rhizobium which is probably involved in the modification of the nodulation Nod factor fatty acyl chain. - Nitrogen fixation protein fixR from Bradyrhizobium japonicum. - Bacillus subtilis protein dltE which is involved in the biosynthesis of D- alanyl-lipoteichoic acid. - Human follicular variant translocation protein 1 (FVT1). - Mouse adipocyte protein p27. - Mouse protein Ke 6. - Maize sex determination protein TASSELSEED 2. - Sarcophaga peregrina 25 Kd development specific protein. - Drosophila fat body protein P6. - A Listeria monocytogenes hypothetical protein encoded in the internalins gene region. - Escherichia coli hypothetical protein yciK. - Escherichia coli hypothetical protein ydfG. - Escherichia coli hypothetical protein yjgI. - Escherichia coli hypothetical protein yjgU. - Escherichia coli hypothetical protein yohF. - Bacillus subtilis hypothetical protein yoxD. - Bacillus subtilis hypothetical protein ywfD. - Bacillus subtilis hypothetical protein ywfH. - Yeast hypothetical protein YIL124w. - Yeast hypothetical protein YIR035c. - Yeast hypothetical protein YIR036c. - Yeast hypothetical protein YKL055c. - Fission yeast hypothetical protein SpAC23D3.11. One of the best conserved regions which includes two perfectly conserved residues, a tyrosine and a lysine has been used as a signature pattern for this family of proteins. The tyrosine residue participates in the catalytic mechanism.

Consensus pattern: [LIVSPADNK]-x(12)-Y-[PSTAGNCV]-[STAGNQCIVM]-[STAGC]-K-{PC}-[SAGFYR]-[LIVMSTAGD]-x(2)-[LIVMFYW]-x(3)- [LIVMFYWGAPTHQ]-[GSACQRHM] [Y is an active site residue]

[1] Joernvall H., Persson B., Krook M., Atrian S., Gonzalez-Duarte R., Jeffery J., Ghosh D. Biochemistry 34:6003-6013(1995).
[2] Villarroya A., Juan E., Egestad B., Joernvall H. Eur. J. Biochem. 180:191-197(1989).
[3] Persson B., Krook M., Joernvall H. Eur. J. Biochem. 200:537-543(1991).
[4] Neidle E.L., Hartnett C., Ornston N.L., Bairoch A., Rekik M., Harayama S. Eur. J. Biochem. 204:113-120(1992).

46. (adh_zinc) Zinc-containing alcohol dehydrogenases signatures

Alcohol dehydrogenase (EC 1.1.1.1) (ADH) catalyzes the reversible oxidation of ethanol to acetaldehyde with the concomitant reduction of NAD [1]. Currently three, structurally and catalytically, different types of alcohol dehydrogenases are known: - Zinc-containing 'long-chain' alcohol dehydrogenases. - Insect-type, or 'short-chain' alcohol dehydrogenases. - Iron-containing alcohol dehydrogenases.Zinc-containing ADH's [2,3] are dimeric or tetrameric enzymes that bind two atoms of zinc per subunit. One of the zinc atom is essential for catalytic activity while the other is not. Both zinc atoms are coordinated by either cysteine or histidine residues; the catalytic zinc is coordinated by two cysteines and one histidine. Zinc-containing ADH's are found in bacteria, mammals, plants, and in fungi. In most species there are more than one isozyme (for example, human have at least six isozymes, yeast have three, etc.). A number of other zinc-dependent dehydrogenases are closely related to zinc ADH [4], these are: - Xylitol dehydrogenase (EC 1.1.1.9) (D-xylulose reductase). - Sorbitol dehydrogenase (EC 1.1.1.14). - Aryl-alcohol dehydrogenase (EC 1.1.1.90) (benzyl alcohol dehydrogenase). - Threonine 3-dehydrogenase (EC 1.1.1.103). - Cinnamyl-alcohol dehydrogenase (EC 1.1.1.195) (CAD) [5]. CAD is a plant enzyme involved in the biosynthesis of lignin. - Galactitol-1-phosphate dehydrogenase (EC 1.1.1.251). - Pseudomonas putida 5-exo-alcohol dehydrogenase (EC 1.1.1.-) [6]. - Escherichia coli starvation sensing protein rspB. - Escherichia coli hypothetical protein yjgB. - Escherichia coli hypothetical protein yjgV. - Escherichia coli hypothetical protein yjjN. - Yeast hypothetical protein YAL060w (FUN49). - Yeast hypothetical protein YAL061w (FUN50). - Yeast hypothetical protein YCR105w. The pattern that has been developed to detect this class of enzymes is based on a conserved region that includes a histidine residue which is the second ligand of the catalytic zinc atom. This family also includes NADP-dependent quinone oxidoreductase (EC 1.6.5.5),an enzyme found in bacteria (gene qor), in yeast and in mammals where, in some species such as rodents, it has been recruited as an eye lens protein and is known as zeta-crystallin [7]. The sequence of quinone oxidoreductase is distantly related to that other zinc-containing alcohol dehydrogenases and it lacks the zinc-ligand residues. The torpedo fish and mammlian synaptic vesicle membrane protein vat-1 is related to qor. A specific pattern has been developed for this subfamily.

Consensus pattern: G-H-E-x(2)-G-x(5)-[GA]-x(2)-[IVSAC] [H is a zinc ligand]
Consensus pattern: [GSD]-[DEQH]-x(2)-L-x(3)-[SA](2)-G-G-x-G-x(4)-Q-x(2)-[KR]-

[1] Branden C.-I., Joernvall H., Eklund H., Furugren B. (In) The Enzymes (3rd edition) 11:104-190(1975).

[2] Joernvall H., Persson B., Jeffery J. Eur. J. Biochem. 167:195-201(1987).

[3] Sun H.-W., Plapp B.V. J. Mol. Evol. 34:522-535(1992).

[4] Persson B., Hallborn J., Walfridsson M., Hahn-Haegerdal B., Keraenen S., Penttilae M., Joernvall H. FEBS Lett. 324:9-14(1993).

[5] Knight M.E., Halpin C., Schuch W. Plant Mol. Biol. 19:793-801(1992).

[6] Koga H., Aramaki H., Yamaguchi E., Takeuchi K., Horiuchi T., Gunsalus I.C. J. Bacteriol. 166:1089-1095(1986).

[7] Joernvall H., Persson B., Du Bois G., Lavers G.C., Chen J.H., Gonzalez P., Rao P.V., Zigler J.S. Jr. FEBS Lett. 322:240-244(1993).

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47. (aldedh) Aldehyde dehydrogenases active sites

Aldehyde dehydrogenases (EC <u>1.2.1.3</u> and EC <u>1.2.1.5</u>) are enzymes which oxidize a wide variety of aliphatic and aromatic aldehydes. In mammals at least four different forms of the enzyme are known [1]: class-1 (or Ald C) a tetrameric cytosolic enzyme, class-2 (or Ald M) a tetrameric mitochondrial enzyme, class-3 (or Ald D) a dimeric cytosolic enzyme, and class IV a microsomal enzyme. Aldehyde dehydrogenases have also been sequenced from fungal and bacterial species. A number of enzymes are known to be evolutionary related to aldehyde dehydrogenases; these enzymes are listed below. - Plants and bacterial betaine-aldehyde dehydrogenase (EC <u>1.2.1.8</u>) [2], an enzyme that catalyzes the last step in the biosynthesis of betaine. - Plants and bacterial NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (EC <u>1.2.1.9</u>). - Escherichia coli succinate-semialdehyde dehydrogenase (NADP+) (EC <u>1.2.1.16</u>) (gene gabD) [3], which reduces succinate semialdehyde into succinate. - Escherichia coli lactaldehyde dehydrogenase (EC <u>1.2.1.22</u>) (gene ald) [4]. - Mammalian succinate semialdehyde dehydrogenase (NAD+) (EC <u>1.2.1.24</u>). - Escherichia coli phenylacetaldehyde dehydrogenase (EC <u>1.2.1.39</u>). - Escherichia coli 5-carboxymethyl-2-hydroxymuconate semialdehyde dehydrogenase (gene hpcC). - Pseudomonas putida 2-hydroxymuconic semialdehyde dehydrogenase [5] (genes dmpC and xylG), an enzyme in the meta-cleavage pathway for the degradation of phenols, cresols and catechol. - Bacterial and mammalian methylmalonate-semialdehyde dehydrogenase (MMSDH) (EC <u>1.2.1.27</u>) [6], an enzyme involved in the distal pathway of valine catabolism. - Yeast delta-1-pyrroline-5-carboxylate dehydrogenase (EC <u>1.5.1.12</u>) [7] (gene PUT2), which converts proline to glutamate. - Bacterial multifunctional putA protein, which contains a delta-1-pyrroline- 5-

carboxylate dehydrogenase domain. - 26G, a garden pea protein of unknown function which is induced by dehydration of shoots [8]. - Mammalian formyltetrahydrofolate dehydrogenase (EC 1.5.1.6) [9]. This is a cytosolic enzyme responsible for the NADP-dependent decarboxylative reduction of 10-formyltetrahydrofolate into tetrahydrofolate. It is an protein of about 900 amino acids which consist of three domains; the C- terminal domain (480 residues) is structurally and functionally related to aldehyde dehydrogenases. - Yeast hypothetical protein YBR006w. - Yeast hypothetical protein YER073w. - Yeast hypothetical protein YHR039c. - Caenorhabditis elegans hypothetical protein F01F1.6.A glutamic acid and a cysteine residue have been implicated in the catalytic activity of mammalian aldehyde dehydrogenase. These residues are conserved in all the enzymes of this family. Two patterns have been derived for this family, one for each of the active site residues.

Consensus pattern: [LIVMFGA]-E-[LIMSTAC]-[GS]-G-[KNLM]-[SADN]-[TAPFV] [E is the active site residue]-

Consensus pattern: [FYLVA]-x(3)-G-[QE]-x-C-[LIVMGSTANC]-[AGCN]-x-[GSTADNEKR] [C is the active site residue

[1] Hempel J., Harper K., Lindahl R. Biochemistry 28:1160-1167(1989).
[2] Weretilnyk E.A., Hanson A.D. Proc. Natl. Acad. Sci. U.S.A. 87:2745-2749(1990).
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[6] Steele M.I., Lorenz D., Hatter K., Park A., Sokatch J.R. J. Biol. Chem. 267:13585-13592(1992).
[7] Krzywicki K.A., Brandriss M.C. Mol. Cell. Biol. 4:2837-2842(1984).
[8] Guerrero F.D., Jones J.T., Mullet J.E. Plant Mol. Biol. 15:11-26(1990).
[9] Cook R.J., Lloyd R.S., Wagner C. J. Biol. Chem. 266:4965-4973(1991).

48. Aldo/keto reductase family signatures

The aldo-keto reductase family [1,2] groups together a number of structurally and functionally related NADPH-dependent oxidoreductases as well as some other proteins. The proteins known to belong to this family are: - Aldehyde reductase (EC 1.1.1.2). - Aldose reductase (EC 1.1.1.21). - 3-alpha-hydroxysteroid dehydrogenase (EC 1.1.1.50), which terminates androgen action by converting 5-alpha-dihydrotestosterone to 3-alpha-androstanediol. - Prostaglandin F synthase (EC 1.1.1.188) which catalyzes the reduction of prostaglandins H2 and D2 to F2-alpha. - D-sorbitol-6-phosphate dehydrogenase (EC 1.1.1.200) from apple. - Morphine 6-dehydrogenase (EC 1.1.1.218) from Pseudomonas putida plasmid pMDH7.2 (gene morA). - Chlordecone reductase (EC 1.1.1.225) which reduces the pesticide chlordecone (kepone) to the corresponding alcohol. - 2,5-diketo-D-gluconic acid reductase (EC 1.1.1.-) which catalyzes the reduction of 2,5-diketogluconic acid to 2-keto-L-gulonic acid, a key intermediate in the production of ascorbic acid. - NAD(P)H-dependent xylose reductase (EC 1.1.1.-) from the yeast Pichia stipitis. This enzyme reduces xylose into xylit. - Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase (EC 1.3.1.20). - 3-oxo-5-beta-steroid 4-dehydrogenase (EC 1.3.99.6) which catalyzes the reduction of delta(4)-3-oxosteroids. - A soybean reductase, which co-acts with chalcone synthase in the formation of 4,2',4'-trihydroxychalcone. - Frog eye lens rho crystallin. - Yeast GCY protein, whose function is not known. - Leishmania major P110/11E protein. P110/11E is a developmentally regulated protein whose abundance is markedly elevated in promastigotes compared with amastigotes. Its exact function is not yet known. - Escherichia coli hypothetical protein yafB. - Escherichia coli hypothetical protein yghE. - Yeast hypothetical protein YBR149w. - Yeast hypothetical protein YHR104w. - Yeast hypothetical protein YJR096w.These proteins have all about 300 amino acid residues. Three consensus patterns have been developed that are specific to this family of proteins. The first one is located in the N-terminal section of these proteins. The second pattern is located in the central section. The third pattern, located in the C-terminal, is centered on a lysine residue whose chemical modification, in aldose and aldehydereductases, affect the catalytic efficiency.

Consensus pattern: G-[FY]-R-[HSAL]-[LIVMF]-D-[STAGC]-[AS]-x(5)-E-x(2)-[LIVM]- G -
Consensus pattern: [LIVMFY]-x(9)-[KREQ]-x-[LIVM]-G-[LIVM]-[SC]-N-[FY]-
Consensus pattern: [LIVM]-[PAIV]-[KR]-[ST]-x(4)-R-x(2)-[GSTAEQK]-[NSL]-x(2)-[LIVMFA] [K is a putative active site residue]-

[1] Bohren K.M., Bullock B., Wermuth B., Gabbay K.H. J. Biol. Chem. 264:9547-9551(1989).
[2] Bruce N.C., Willey D.L., Coulson A.F.W., Jeffery J. Biochem. J. 299:805-811(1994).

49. Alpha amylase. This family is classified as family 13 of the glycosyl hydrolases. The structure is an 8 stranded alpha/beta barrel, interrupted by a ~70 a.a. calcium-binding domain protruding between beta strand 3 and alpha helix 3, and a carboxyl-terminal Greek key beta-barrel domain.

[1] Larson SB, Greenwood A, Cascio D, Day J, McPherson A, J Mol Biol 1994;235:1560-1584.

50. Aminotransferases class-I pyridoxal-phosphate attachment site
Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-I, currently consists of the following enzymes: - Aspartate aminotransferase (AAT) (EC <u>2.6.1.1</u>). AAT catalyzes the reversible transfer of the amino group from L-aspartate to 2-oxoglutarate to form oxaloacetate and L-glutamate. In eukaryotes, there are two AAT isozymes: one is located in the mitochondrial matrix, the second is cytoplasmic. In prokaryotes, only one form of AAT is found (gene aspC). - Tyrosine aminotransferase (EC <u>2.6.1.5</u>) which catalyzes the first step in tyrosine catabolism by reversibly transferring its amino group to 2- oxoglutarate to form 4-hydroxyphenylpyruvate and L-glutamate. - Aromatic aminotransferase (EC <u>2.6.1.57</u>) involved in the synthesis of Phe, Tyr, Asp and Leu (gene tyrB). - 1-aminocyclopropane-1-carboxylate synthase (EC <u>4.4.1.14</u>) (ACC synthase) from plants. ACC synthase catalyzes the first step in ethylene biosynthesis. - Pseudomonas denitrificans cobC, which is involved in cobalamin biosynthesis. - Yeast hypothetical protein YJL060w.The sequence around the pyridoxal-phosphate attachment site of this class of enzyme is sufficiently conserved to allow the creation of a specific pattern.

Consensus pattern: [GS]-[LIVMFYTAC]-[GSTA]-K-x(2)-[GSALVN]-[LIVMFA]-x-[GNAR]- x-R-[LIVMA]-[GA] [K is the pyridoxal-P attachment site]

[1] Bairoch A. Unpublished observations (1992).

[2] Sung M.H., Tanizawa K., Tanaka H., Kuramitsu S., Kagamiyama H., Hirotsu K., Okamoto A., Higuchi T., Soda K. J. Biol. Chem. 266:2567-2572(1991).

51. Aminotransferases class-II pyridoxal-phosphate attachment site

Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1] into subfamilies. One of these, called class-II, currently consists of the following enzymes: - Glycine acetyltransferase (EC 2.3.1.29), which catalyzes the addition of acetyl-CoA to glycine to form 2-amino-3-oxobutanoate (gene kbl). - 5-aminolevulinic acid synthase (EC 2.3.1.37) (delta-ALA synthase), which catalyzes the first step in heme biosynthesis via the Shemin (or C4) pathway, i.e. the addition of succinyl-CoA to glycine to form 5-aminolevulinate. - 8-amino-7-oxononanoate synthase (EC 2.3.1.47) (7-KAP synthetase), a bacterial enzyme (gene bioF) which catalyzes an intermediate step in the biosynthesis of biotin: the addition of 6-carboxy-hexanoyl-CoA to alanine to form 8-amino-7-oxononanoate. - Histidinol-phosphate aminotransferase (EC 2.6.1.9), which catalyzes the eighth step in histidine biosynthetic pathway: the transfer of an amino group from 3-(imidazol-4-yl)-2-oxopropyl phosphate to glutamic acid to form histidinol phosphate and 2-oxoglutarate. - Serine palmitoyltransferase (EC 2.3.1.50) from yeast (genes LCB1 and LCB2), which catalyzes the condensation of palmitoyl-CoA and serine to form 3- ketosphinganine.The sequence around the pyridoxal-phosphate attachment site of this class of enzyme is sufficiently conserved to allow the creation of a specific pattern

Consensus pattern: T-[LIVMFYW]-[STAG]-K-[SAG]-[LIVMFYWR]-[SAG]-x(2)-[SAG] [K is the pyridoxal-P attachment site]-

[1] Bairoch A. Unpublished observations (1991).

52. Aminotransferases class-III pyridoxal-phosphate attachment site

Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-III, currently consists of the following enzymes: - Acetylornithine aminotransferase (EC 2.6.1.11) which catalyzes the transfer of an amino group from acetylornithine to alpha-ketoglutarate, yielding N-acetyl-glutamic-5-semi-aldehyde and glutamic acid. - Ornithine aminotransferase (EC 2.6.1.13), which catalyzes the transfer of an amino group from ornithine to alpha-ketoglutarate, yielding glutamic-5- semi-aldehyde and glutamic acid. - Omega-amino acid--pyruvate aminotransferase (EC 2.6.1.18), which catalyzes transamination between a variety of omega-amino acids, mono- and diamines, and pyruvate. It plays a pivotal role in omega amino acids metabolism. - 4-aminobutyrate aminotransferase (EC 2.6.1.19) (GABA transaminase), which catalyzes the transfer of an amino group from GABA to alpha-ketoglutarate, yielding succinate semialdehyde and glutamic acid. - DAPA aminotransferase (EC 2.6.1.62), a bacterial enzyme (gene bioA) which catalyzes an intermediate step in the biosynthesis of biotin, the transamination of 7-keto-8-aminopelargonic acid (7-KAP) to form 7,8- diaminopelargonic acid (DAPA). - 2,2-dialkylglycine decarboxylase (EC 4.1.1.64), a Pseudomonas cepacia enzyme (gene dgdA) that catalyzes the decarboxylating amino transfer of 2,2-dialkylglycine and pyruvate to dialkyl ketone, alanine and carbon dioxide. - Glutamate-1-semialdehyde aminotransferase (EC 5.4.3.8) (GSA). GSA is the enzyme involved in the second step of porphyrin biosynthesis, via the C5 pathway. It transfers the amino group on carbon 2 of glutamate-1- semialdehyde to the neighbouring carbon, to give delta-aminolevulinic acid. - Bacillus subtilis aminotransferase yhxA. - Bacillus subtilis aminotransferase yodT. - Haemophilus influenzae aminotransferase HI0949. - Caenorhabditis elegans aminotransferase T01B11.2.The sequence around the pyridoxal-phosphate attachment site of this class ofenzyme is sufficiently conserved to allow the creation of a specific pattern.

Consensus pattern: [LIVMFYWC](2)-x-D-E-[IVA]-x(2)-G-[LIVMFAGC]-x(0,1)-[RSACLI]-x-[GSAD]-x(12,16)-D-[LIVMFC]-[LIVMFYSTA]-x(2)- [GSA]-K-x(3)-[GSTADNV]-[GSAC] [K is the pyridoxal-P attachment site]-

[1] Bairoch A. Unpublished observations (1992).[2] Yonaha K., Nishie M., Aibara S. J. Biol. Chem. 267:12506-12510(1992).

53. Ank repeat. There's no clear separation between noise and signal on the HMM search Ankyrin repeats generally consist of a beta, alpha, alpha, beta order of secondary structures. The repeats associate to form a higher order structure.

[1] A, Holak TA, FEBS Lett 1997;401:127-132.

[2] Lux SE, John KM, Bennett V, Nature 1990;345:736-739.

54. Aminotransferases class-IV signature

Aminotransferases share certain mechanistic features with other pyridoxal-phosphate dependent enzymes, such as the covalent binding of the pyridoxal-phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-IV, currently consists of the following enzymes:

- Branched-chain amino-acid aminotransferase (EC <u>2.6.1.42</u>) (transaminase B), a bacterial (gene ilvE) and eukaryotic enzyme which catalyzes the reversible transfer of an amino group from 4-methyl-2-oxopentanoate to glutamate, to form leucine and 2-oxoglutarate.
- D-alanine aminotransferase (EC <u>2.6.1.21</u>). A bacterial enzyme which catalyzes the transfer of the amino group from D-alanine (and other D-amino acids) to 2-oxoglutarate, to form pyruvate and D-aspartate.
- 4-amino-4-deoxychorismate (ADC) lyase (gene pabC). A bacterial enzyme that converts ADC into 4-aminobenzoate (PABA) and pyruvate.

The above enzymes are proteins of about 270 to 415 amino-acid residues that share a few regions of sequence similarity. Surprisingly, the best-conserved region does not include the lysine residue to which the pyridoxal-phosphategroup is known to be attached, in ilvE. The region that has been selected as a signature pattern is located some 40 residues at the C-terminus side of the PlP-lysine

Consensus pattern: E-x-[STAGCI]-x(2)-N-[LIVMFAC]-[FY]-x(6,12)-[LIVMF]-x-T- x(6,8)-[LIVM]-x-[GS]-[LIVM]-x-[KR]-

[1] Green J.M., Merkel W.K., Nichols B.P. J. Bacteriol. 174:5317-5323(1992).

[2] Bairoch A. Unpublished observations (1992).

55. Aminotransferases class-V pyridoxal-phosphate attachment site

Aminotransferases share certain mechanistic features with other pyridoxal- phosphate dependent enzymes, such as the covalent binding of the pyridoxal- phosphate group to a lysine residue. On the basis of sequence similarity, these various enzymes can be grouped [1,2] into subfamilies. One of these, called class-V, currently consists of the following enzymes: - Phosphoserine aminotransferase (EC 2.6.1.52), an enzyme which catalyzes the reversible interconversion of phosphoserine and 2-oxoglutarate to 3-phosphonooxypyruvate and glutamate. It is required both in the major phosphorylated pathway of serine biosynthesis and in pyridoxine biosynthesis. The bacterial enzyme (gene serC) is highly similar to a rabbit endometrial progesterone-induced protein (EPIP), which is probably a phosphoserine aminotransferase [3]. - Serine--glyoxylate aminotransferase (EC 2.6.1.45) (SGAT) (gene sgaA) from Methylobacterium extorquens. - Serine--pyruvate aminotransferase (EC 2.6.1.51). This enzyme also acts as an alanine--glyoxylate aminotransferase (EC 2.6.1.44). In vertebrates, it is located in the peroxisomes and/or mitochondria. - Isopenicillin N epimerase (gene cefD). This enzyme is involved in the biosynthesis of cephalosporin antibiotics and catalyzes the reversible isomerization of isopenicillin N and penicillin N. - NifS, a protein of the nitrogen fixation operon of some bacteria and cyanobacteria. The exact function of nifS is not yet known. A highly similar protein has been found in fungi (gene NFS1 or SPL1). - The small subunit of cyanobacterial soluble hydrogenase (EC 1.12.-.-). - Hypothetical protein ycbU from Bacillus subtilis. - Hypothetical protein YFL030w from yeast. The sequence around the pyridoxal-phosphate attachment site of this class of enzyme is sufficiently conserved to allow the creation of a specific pattern.

Consensus pattern: [LIVFYCHT]-[DGH]-[LIVMFYAC]-[LIVMFYA]-x(2)-[GSTAC]-[GSTA]- [HQR]-K-x(4,6)-G-x-[GSAT]-x-[LIVMFYSAC] [K is the pyridoxal-P attachment site]-

[1] Ouzounis C., Sander C. FEBS Lett. 322:159-164(1993).

[2] Bairoch A. Unpublished observations (1992).

[3] van der Zel A., Lam H.-M., Winkler M.E. Nucleic Acids Res. 17:8379-8379(1989).

56. Annexins repeated domain signature

Annexins [1 to 6] are a group of calcium-binding proteins that associate reversibly with membranes. They bind to phospholipid bilayers in the presence of micromolar free calcium concentration. The binding is specific for calcium and for acidic phospholipids. Annexins have been claimed to be involved in cytoskeletal interactions, phospholipase inhibition, intracellular signalling, anticoagulation, and membrane fusion. Each of these proteins consist of an N-terminal domain of variable length followed by four or eight copies of a conserved segment of sixty one residues. The repeat (sometimes known as an 'endonexin fold') consists of five alpha-helices that are wound into a right-handed superhelix [7].The proteins known to belong to the annexin family are listed below: - Annexin I (Lipocortin 1) (Calpactin 2) (p35) (Chromobindin 9). - Annexin II (Lipocortin 2) (Calpactin 1) (Protein I) (p36) (Chromobindin 8). - Annexin III (Lipocortin 3) (PAP-III). - Annexin IV (Lipocortin 4) (Endonexin I) (Protein II) (Chromobindin 4). - Annexin V (Lipocortin 5) (Endonexin 2) (VAC-alpha) (Anchorin CII) (PAP-I). - Annexin VI (Lipocortin 6) (Protein III) (Chromobindin 20) (p68) (p70). This is the only known annexin that contains 8 (instead of 4) repeats. - Annexin VII (Synexin). - Annexin VIII (Vascular anticoagulant-beta) (VAC-beta). - Annexin IX from Drosophila. - Annexin X from Drosophila. - Annexin XI (Calcyclin-associated annexin) (CAP-50). - Annexin XII from Hydra vulgaris. - Annexin XIII (Intestine-specific annexin) (ISA).The signature pattern for this domain spans positions 9 to 61 of the repeatand includes the only perfectly conserved residue (an arginine in position 22)-

Consensus pattern: [TG]-[STV]-x(8)-[LIVMF]-x(2)-R-x(3)-[DEQNH]-x(7)-[IFY]- x(7)-[LIVMF]-x(3)-[LIVMF]-x(11)-[LIVMFA]-x(2)-[LIVMF]-

[1] Raynal P., Pollard H.B. Biochim. Biophys. Acta 1197:63-93(1994).
[2] Barton G.J., Newman R.H., Freemont P.S., Crumpton M.J. Eur. J. Biochem. 198:749-760(1991).
[3] Burgoyne R.D., Geisow M.J. Cell Calcium 10:1-10(1989).
[4] Haigler H.T., Fitch J.M., Jones J.M., Schlaepfer D.D. Trends Biochem. Sci. 14:48-50(1989).

[5] Klee C.B. Biochemistry 27:6645-6653(1988).
[6] Smith P.D., Moss S.E. Trends Genet. 10:241-246(1994).
[7] Huber R., Roemisch J., Paques E.-P. EMBO J. 9:3867-3874(1990).
[8] Fiedler K., Simons K. Trends Biochem. Sci. 20:177-178(1995).

57. (arf_1) ADP-ribosylation factors family signature

ADP-ribosylation factors (ARF) [1,2,3,4] are 20 Kd GTP-binding proteins involved in protein trafficking. They may modulate vesicle budding and uncoating within the Golgi apparatus. ARF's also act as allosteric activators of cholera toxin ADP-ribosyltransferase activity. They are evolutionary conserved and present in all eukaryotes. At least six forms of ARF are present in mammals and three in budding yeast. The ARF family also includes proteins highly related to ARF's but which lack the cholera toxin cofactor activity, they are collectively known as ARL's (ARF-like).ARD1 is a 64 Kd mammalian protein of unknown biological function that contains an ARF domain at its C-terminal extremity. Proteins from the ARF family are generally included in the RAS 'superfamily' of small GTP-binding proteins [5], but they are only slightly related to the other RAS proteins. They also differ from RAS proteins in that they lack cysteine residues at their C-termini and are therefore not subject to prenylation. The ARFs are N-terminally myristoylated (the ARLs have not yet been shown to be modified in such a fashion). A conserved region in the C-terminal part of ARF's and ARL's has been selected as a signature pattern.

Consensus pattern: [HRQT]-x-[FYWI]-x-[LIVM]-x(4)-A-x(2)-G-x(2)-[LIVM]-x(2)- [GSA]-[LIVMF]-x-[WK]-[LIVM]-

Note: proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop) (see <PDOC00017

[1] Boman A.L., Kahn R.A. Trends Biochem. Sci. 20:147-150(1995).
[2] Moss J., Vaughan M. Cell. Signal. 4.367-399(1993).
[3] Moss J., Vaughan M. Prog. Nucleic Acid Res. Mol. Biol. 45:47-65(1993).
[4] Amor J.C., Harrison D.H., Kahn R.A., Ringe D. Nature 372:704-708(1994).
[5] Valencia A., Chardin P., Wittinghofer A., Sander C. Biochemistry 30:4637-4648(1991).

(arf_2) ATP/GTP-binding site motif A (P-loop)

From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix. This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5].There are numerous ATP- or GTP-binding proteins in which the P-loop is found. A number of protein families for which the relevance of the presence of such motif has been noted are listed below: - ATP synthase alpha and beta subunits (see <PDOC00137>). - Myosin heavy chains. - Kinesin heavy chains and kinesin-like proteins (see <PDOC00343>). - Dynamins and dynamin-like proteins (see <PDOC00362>). - Guanylate kinase (see <PDOC00670>). - Thymidine kinase (see <PDOC00524>). - Thymidylate kinase (see <PDOC01034>). - Shikimate kinase (see <PDOC00868>). - Nitrogenase iron protein family (nifH/frxC) (see <PDOC00580>). - ATP-binding proteins involved in 'active transport' (ABC transporters) [7] (see <PDOC00185>). - DNA and RNA helicases [8,9,10]. - GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.). - Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.). - Nuclear protein ran (see <PDOC00859>). - ADP-ribosylation factors family (see <PDOC00781>). - Bacterial dnaA protein (see <PDOC00771>). - Bacterial recA protein (see <PDOC00131>). - Bacterial recF protein (see <PDOC00539>). - Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.). - DNA mismatch repair proteins mutS family (See <PDOC00388>). - Bacterial type II secretion system protein E (see <PDOC00567>).Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the glycolytic kinases. In other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved for adenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.

Consensus pattern: [AG]-x(4)-G-K-[ST]-

[1] Walker J.E., Saraste M., Runswick M.J., Gay N.J. EMBO J. 1:945-951(1982).

[2] Moller W., Amons R. FEBS Lett. 186:1-7(1985).

[3] Fry D.C., Kuby S.A., Mildvan A.S. Proc. Natl. Acad. Sci. U.S.A. 83:907-911(1986).

[4] Dever T.E., Glynias M.J., Merrick W.C. Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).

[5] Saraste M., Sibbald P.R., Wittinghofer A. Trends Biochem. Sci. 15:430-434(1990).

[6] Koonin E.V. J. Mol. Biol. 229:1165-1174(1993).

[7] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg. Biomembr. 22:571-592(1990).

[8] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).

[9] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).

[10] Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M. Nucleic Acids Res. 17:4713-4730(1989).

58. Arginase family signatures

The following enzymes have been shown [1] to be evolutionary related: - Arginase (EC 3.5.3.1), a ubiquitous enzyme which catalyzes the degradation of arginine to ornithine and urea [2]. - Agmatinase (EC 3.5.3.11) (agmatine ureohydrolase), a prokaryotic enzyme (gene speB) that catalyzes the hydrolysis of agmatine into putrescine and urea. - Formiminoglutamase (EC 3.5.3.8) (formiminoglutamate hydrolase), a prokaryotic enzyme (gene hutG) that hydrolyzes N-formimino-glutamate into glutamate and formamide. - Hypothetical proteins from methanogenic archaebacteria. These enzymes are proteins of about 300 amino-acid residues. Three conserved regions that contain charged residues which are involved in the binding of the two manganese ions [3] can be used as signature patterns.-

Consensus pattern: [LIVMF]-G-G-x-H-x-[LIVMT]-[STAV]-x-[PAG]-x(3)-[GSTA] [H binds manganese]-

Consensus pattern: [LIVM](2)-x-[LIVMFY]-D-[AS]-H-x-D [The two D's and the H bind manganese]-

Consensus pattern: [ST]-[LIVMFY]-D-[LIVM]-D-x(3)-[PAQ]-x(3)-P-[GSA]-x(7)-G [The two D's bind manganese]

[1] Ouzounis C., Kyrpides N.C. J. Mol. Evol. 39:101-104(1994).

[2] Jenkinson C.P., Grody W.W., Cederbaum S.D. Comp. Biochem. Physiol. 114B:107-132(196).

[3] Kanyo Z.F., Scolnick L.R., Ash D.E., Christianson D.W. Nature 383:554-557(1996).

59. (asp) Eukaryotic and viral aspartyl proteases active site

Aspartyl proteases, also known as acid proteases, (EC 3.4.23.-) are a widely distributed family of proteolytic enzymes [1,2,3] known to exist invertebrates, fungi, plants, retroviruses and some plant viruses. Aspartate proteases of eukaryotes are monomeric enzymes which consist of two domains. Each domain contains an active site centered on a catalytic aspartyl residue.The two domains most probably evolved from the duplication of an ancestral gene encoding a primordial domain. Currently known eukaryotic aspartyl proteases are: - Vertebrate gastric pepsins A and C (also known as gastricsin). - Vertebrate chymosin (rennin), involved in digestion and used for making cheese. - Vertebrate lysosomal cathepsins D (EC 3.4.23.5) and E (EC 3.4.23.34). - Mammalian renin (EC 3.4.23.15) whose function is to generate angiotensin I from angiotensinogen in the plasma. - Fungal proteases such as aspergillopepsin A (EC 3.4.23.18), candidapepsin (EC 3.4.23.24), mucoropepsin (EC 3.4.23.23) (mucor rennin), endothiapepsin (EC 3.4.23.22), polyporopepsin (EC 3.4.23.29), and rhizopuspepsin (EC 3.4.23.21). - Yeast saccharopepsin (EC 3.4.23.25) (proteinase A) (gene PEP4). PEP4 is implicated in posttranslational regulation of vacuolar hydrolases. - Yeast barrier pepsin (EC 3.4.23.35) (gene BAR1); a protease that cleaves alpha-factor and thus acts as an antagonist of the mating pheromone. - Fission yeast sxa1 which is involved in degrading or processing the mating pheromones. Most retroviruses and some plant viruses, such as badnaviruses, encode for anaspartyl protease which is an homodimer of a chain of about 95 to 125 amino acids. In most retroviruses, the protease is encoded as a segment of apolyprotein which is cleaved during the maturation process of the virus. It is generally part of the pol polyprotein and, more rarely, of the gagpolyprotein. Conservation of the sequence around the two aspartates of eukaryotic aspartyl proteases and around the single active site of the viral proteases allows us to develop a single signature pattern for both groups of protease.

Consensus pattern: [LIVMFGAC]-[LIVMTADN]-[LIVFSA]-D-[ST]-G-[STAV]-[STAPDENQ]- x-[LIVMFSTNC]-x-[LIVMFGTA] [D is the active site residue]

09555352.051501

Note: these proteins belong to families A1 and A2 in the classification of peptidases [4,E1

[1] Foltmann B. Essays Biochem. 17:52-84(1981).
[2] Davies D.R. Annu. Rev. Biophys. Chem. 19:189-215(1990).
[3] Rao J.K.M., Erickson J.W., Wlodawer A. Biochemistry 30:4663-4671(1991).
[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:105-120(1995).

60. (BIRA) Biotin repressor

[1] Wilson KP, Shewchuk LM, Brennan RG, Otsuka AJ, Matthews BW; Proc Natl Acad Sci USA 1992;89:9257-9261.

09545355.051502

61. BTB/POZ domain

The BTB (for BR-C, ttk and bab) [1] or POZ (for Pox virus and Zinc finger)[2] domain is present near the N-terminus of a fraction of zinc finger (zf-C2H2) proteins and in proteins that contain the Kelch motif such as Kelch and a family of pox virus proteins. The BTB/POZ domain mediates homomeric dimerisation and in some instances heteromeric dimerisation [2].The structure of the dimerised PLZF BTB/POZ domain has been solved and consists of a tightly intertwined homodimer. The central scaffolding of the protein is made up of a cluster of alpha-helices flanked by short beta-sheets at both the top and bottom of the molecule [3]. POZ domains from several zinc finger proteins have been shown to mediate transcriptional repression and to interact with components of histone deacetylase co-repressor complexes including N-CoR and SMRT [4,5,6]. The POZ or BTB domain is also known as BR-C/Ttk or ZiN

[1] Zollman S, Godt D, Prive GG, Couderc JL, Laski FA; Proc Natl Acad Sci U S A 1994;91:10717-10721.
[2]Bardwell VJ, Treisman R; Genes Dev 1994;8:1664-1677.
[3] Ahmad KF, Engel CK, Prive GG; Proc Natl Acad Sci U S A 1998;95:12123-12128.
[4] Deweindt C, Albagli O, Bernardin F, Dhordain P, Quief S, Lantoine D, Kerckaert JP, Leprince D; Cell Growth Differ 1995;6:1495-1503.
[5] Huynh KD, Bardwell VJ; Oncogene 1998;17:2473-2484.

[6] Wong CW, Privalsky ML; J Biol Chem 1998;273:27695-27702.

62. (Bac GSPproteins) Bacterial type II secretion system protein D signature

A number of bacterial proteins, some of which are involved in a general secretion pathway (GSP) for the export of proteins (also called the type II pathway) [1 to 5], have been found to be evolutionary related. These proteins are listed below: - The 'D' protein from the GSP operon of: Aeromonas (gene exeD); Erwinia (gene outD); Escherichia coli (gene yheF), Klebsiella pneumoniae (gene pulD); Pseudomonas aeruginosa (gene xcpQ); Vibrio cholerae (gene epsD) and Xanthomonas campestris (gene xpsD). - comE from Haemophilus influenzae, involved in competence (DNA uptake). - pilQ from Pseudomonas aeruginosa, which is essential for the formation of the pili. - hofQ (hopQ) from Escherichia coli. - hrpH from Pseudomonas syringae, which is involved in the secretion of a proteinaceous elicitor of the hypersensitivity response in plants. - hrpA1 from Xanthomonas campestris pv. vesicatoria, which is also involved in the hypersensitivity response. - mxiD from Shigella flexneri which is involved in the secretion of the Ipa invasins which are necessary for penetration of intestinal epithelial cells. - omc from Neisseria gonorrhoeae. - yssC from Yersinia enterocolitica virulence plasmid pYV, which seems to be required for the export of the Yop virulence proteins. - The gpIV protein from filamentous phages such as f1, ike, or m13. GpIV is said to be involved in phage assembly and morphogenesis. These proteins all seem to start with a signal sequence and are thought to be integral proteins in the outer membrane. As a signature pattern a conserved region in the C-terminal section of these proteins has been selected

Consensus pattern: [GR]-[DEQKG]-[STVM]-[LIVMA](3)-[GA]-G-[LIVMFY]-x(11)-[LIVM]-P-[LIVMFYWGS]-[LIVMF]-[GSAE]-x-[LIVM]-P- [LIVMFYW](2)-x(2)-[LV]-F

[1] Salmond G.P.C., Reeves P.J. Trends Biochem. Sci. 18:7-12(1993).

[2] Reeves P.J., Whitcombe D., Wharam S., Gibson M., Allison G., Bunce N., Barallon R., Douglas P., Mulholland V., Stevens S., Walker S., Salmond G.P.C. Mol. Microbiol. 8:443-456(1993).

[3] Martin P.R., Hobbs M., Free P.D., Jeske Y., Mattick J.S. Mol. Microbiol. 9:857-868(1993).

[4] Hobbs M., Mattick J.S. Mol. Microbiol. 10:233-243(1993).

[5] Genin S., Boucher C.A. Mol. Gen. Genet. 243:112-118(1994).

63. (Bac globin) Protozoan/cyanobacterial globins signature

Globins are heme-containing proteins involved in binding and/or transporting oxygen [1]. Almost all globins belong to a large family (see <PDOC00793>), the only exceptions are the following proteins which form a family of their own[2,3]: - Monomeric hemoglobins from the protozoan Paramecium caudatum, Tetrahymena pyriformis and Tetrahymena thermophila. - Cyanoglobin from the cyanobacteria Nostoc commune. - Globins LI637 and LI410 from the chloroplast of the alga Chlamydomonas eugametos. - Mycobacterium tuberculosis hypothetical protein MtCY48.23.These proteins contain a conserved histidine which could be involved in heme-binding. As a signature pattern, a conserved region that ends with this residue was used

Consensus pattern: F-[LF]-x(5)-G-[PA]-x(4)-G-[KRA]-x-[LIVM]-x(3)-H-

[1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).

[2] Takagi T. Curr. Opin. Struct. Biol. 3:413-418(1993).

[3] Couture M., Chamberland H., St-Pierre B., Lafontaine J., Guertin M.; Mol. Gen. Genet. 243:185-197(1994).

64. Band 7 protein family signature

Mammalian band 7 protein [1] (also known as 7.2B or stomatin) is an integral membrane phosphoprotein of red blood cells thought to regulate cation conductance by interacting with other proteins of the junctional complex of the membrane skeleton. Structurally, band 7 is evolutionary related to the following proteins: - Caenorhabditis elegans protein mec-2 [2]. Mec-2 positively regulates the activity of the putative mechanosensory transduction channel. It may links the mechanosensory channel and the microtubule cytoskeleton of the touch receptor neurons. - Caenorhabditis elegans proteins sto-1 to sto-4. - Caenorhabditis elegans protein unc-1. - Escherichia coli hypothetical protein ybbK. - Mycobacterium tuberculosis hypothetical protein MtCY277.09. - Synechocystis strain PCC 6803 hypothetical protein slr1128. - Methanococcus jannaschii hypothetical protein MJ0827.Structurally all these proteins consist of a short N-terminal domain which is followed by a transmembrane region and a variable size (from 170 to 350residues) C-terminal domain .As a signature pattern, a conserved region located about 110residues after the transmembrane domain was selected

Consensus pattern: R-x(2)-[LIV]-[SAN]-x(6)-[LIV]-D-x(2)-T-x(2)-W-G-[LIV]- [KRH]-[LIV]-x-[KR]-[LIV]-E-[LIV]-[KR]-

[1] Gallagher P.G., Forget B.G. J. Biol. Chem. 270:26358-26363(1995).
[2] Huang M., Gu G., Ferguson E.L., Chalfie M. Nature 378:292-295(1995).

65. Barwin domain signatures

Barwin [1] is a barley seed protein of 125 residues that binds weakly a chitinanalog. It contains six cysteines involved in disulfide bonds, as shown in the following schematic representation.
+---------------+ | ***** | ****
xxxxxxxxxxxxxxxCxxxxxxxxxxCxxxxCxCxxxxxxxxxCxxxxxxxxxxxxxxxxxxCx | | | | +---------------+ +--------------------------+'C': conserved cysteine involved in a disulfide bond.'*': position of the patterns. Barwin is closely related to the following proteins: - Hevein, a wound-induced protein found in the latex of rubber trees. - HEL, an Arabidopsis thaliana hevein-like protein [2]. - Win1 and win2, two wound-induced proteins from potato. - Pathogenesis-related protein 4 from tobacco. Hevein and the win1/2 proteins consist of an N-terminal chitin-binding domain followed by a barwin-like C-terminal domain. Barwin and its related proteins could be involved in a defense mechanism in plants. As signature patterns, two highly conserved regions that contain some of the cysteines were selected

Consensus pattern: C-G-[KR]-C-L-x-V-x-N [The two C's are involved in disulfide bonds]-
Consensus pattern: V-[DN]-Y-[EQ]-F-V-[DN]-C [C is involved in a disulfide bond]-

[1] Svensson B., Svendsen I., Hoejrup P., Roepstorff P., Ludvigsen S., Poulsen F.M. Biochemistry 31:8767-8770(1992).

[2] Potter S., Uknes S., Lawton K., Winter A.M., Chandler D., Dimaio J., Novitzky R., Ward E., Ryals J. Mol. Plant Microbe Interact. 6:680-685(1993).

66. (Bowman-Birk leg) Bowman-Birk serine protease inhibitors family signature PROSITE cross-reference(s). The Bowman-Birk inhibitor family [1] is one of the numerous families of serine proteinase inhibitors. As it can be seen in the schematic representation, they have a duplicated structure and generally possess two distinct inhibitory sites:

```
   +---------------------------------------------+
   |   +-----+     +-------+    +-----+      |
   |    |   |      |    |   |    |
  xxCCxxCxxCxx#xxCxxCxxxxCxxxCxxxCxxxxCxx#xxCxxCxxCxxCxx
    | |       |*******|****          | |
    | |       |     |          | |
    +--|-----------+     +----------------+ |
      +--------------------------------------+

   <----------------70 residues------------------->
```

'C': conserved cysteine involved in a disulfide bond.
'#': active site residue.
'*': position of the pattern.

These inhibitors are found in the seeds of all leguminous plants as well as in cereal grains. In cereals they exist in two forms, one of which is a duplication of the basic structure shown above [2]. The pattern that was developed to pick up sequences belonging to this family of inhibitors is in the central part of the domain and includes four cysteines.

Consensus pattern C-x(5,6)-[DENQKRHSTA]-C-[PASTDH]-[PASTDK]-[ASTDV]-C-[NDKS]-[DEKRHSTA]-C [The four C's are involved in disulfide bonds] Note this pattern can be found twice in some duplicated cereal inhibitors.

[1] Laskowski M., Kato I. Annu. Rev. Biochem. 49:593-626(1980).

[2] Tashiro M., Hashino K., Shiozaki M., Ibuki F., Maki Z. J. Biochem. 102:297-306(1987).

67. Pathogenesis-related protein Bet v I family signature

A number of plant proteins, which all seem to be involved in pathogen defense response, are structurally related [1,2,3]. These proteins are:

- Bet v I, the major pollen allergen from white birch. Bet v I is the main cause of type I allergic reactions in Europe, North America and USSR.
- Aln g I, the major pollen allergen from alder.
- Api G I, the major allergen from celery.
- Car b I, the major pollen allergen from hornbeam.
- Cor a I, the major pollen allergen from hazel.
- Mal d I, the major pollen allergen from apple.
- Asparagus wound-induced protein AoPR1.
- Kidney bean pathogenesis-related proteins 1 and 2.
- Parsley pathogenesis-related proteins PR1-1 and PR1-3.
- Pea disease resistance response proteins pI49, pI176 and DRRG49-C.
- Pea abscisic acid-responsive proteins ABR17 and ABR18.
- Potato pathogenesis-related proteins STH-2 and STH-21.
- Soybean stress-induced protein SAM22.

These proteins are thought to be intracellularly located. They contain from 155 to 160 amino acid residues. As a signature pattern, a conserved region located in the third quarter of these proteins has been selected

Consensus pattern: G-x(2)-[LIVMF]-x(4)-E-x(2)-[CSTAEN]-x(8,9)-[GND]-G-[GS]- [CS]-x(2)-K-x(4)-[FY]-

[1] Breiteneder H., Pettenburger K., Bito A., Valenta R., Kraft D., Rumpold H., Scheiner O., Breitenbach M. EMBO J. 8:1935-1938(1989).

[2] Crowell D., John M.E., Russell D., Amasino R.M. Plant Mol. Biol. 18:459-466(1992).

[3] Warner S.A.J., Scott R., Draper J. Plant Mol. Biol. 19:555-561(1992).

68. bZIP transcription factors basic domain signature

The bZIP superfamily [1,2,] of eukaryotic DNA-binding transcription factors groups together proteins that contain a basic region mediating sequence-specific DNA-binding followed by a leucine zipper required for dimerization. This family is quite large, therefore only a parital list of some representative members appears here. - Transcription factor AP-1, which binds selectively to enhancer elements in the cis control regions of SV40 and metallothionein IIA. AP-1, also known as c-jun, is the cellular homolog of the avian sarcoma virus 17 (ASV17) oncogene v-jun. - Jun-B and jun-D, probable transcription factors which are highly similar to jun/AP-1. - The fos protein, a proto-oncogene that forms a non-covalent dimer with c-jun. - The fos-related proteins fra-1, and fos B. - Mammalian cAMP response element (CRE) binding proteins CREB, CREM, ATF-1, ATF-3, ATF-4, ATF-5, ATF-6 and LRF-1. - Maize Opaque 2, a trans-acting transcriptional activator involved in the regulation of the production of zein proteins during endosperm. - Arabidopsis G-box binding factors GBF1 to GBF4, Parsley CPRF-1 to CPRF-3, Tobacco TAF-1 and wheat EMBP-1. All these proteins bind the G-box promoter elements of many plant genes. - Drosophila protein Giant, which represses the expression of both the kruppel and knirps segmentation gap genes. - Drosophila Box B binding factor 2 (BBF-2), a transcriptional activator that binds to fat body-specific enhancers of alcohol dehydrogenase and yolk protein genes. - Drosophila segmentation protein cap'n'collar (gene cnc), which is involved in head morphogenesis. - Caenorhabditis elegans skn-1, a developmental protein involved in the fate of ventral blastomeres in the early embryo. - Yeast GCN4 transcription factor, a component of the general control system that regulates the expression of amino acid-synthesizing enzymes in response to amino acid starvation, and the related Neurospora crassa cpc-1 protein. - Neurospora crassa cys-3 which turns on the expression of structural genes which encode sulfur-catabolic enzymes. - Yeast MET28, a transcriptional activator of sulfur amino acids metabolism. - Yeast PDR4 (or YAP1), a transcriptional activator of the genes for some oxygen detoxification enzymes. - Epstein-Barr virus trans-activator protein BZLF1.-

Consensus pattern: [KR]-x(1,3)-[RKSAQ]-N-x(2)-[SAQ](2)-x-[RKTAENQ]-x-R-x-[RK]-

[1] Hurst H.C. Protein Prof. 2:105-168(1995).[2] Ellenberger T. Curr. Opin. Struct. Biol. 4:12-21(1994).

69. Biotin-requiring enzymes attachment site

Biotin, which plays a catalytic role in some carboxyl transfer reactions, is covalently attached, via an amide bond, to a lysine residue in enzymes requiring this coenzyme [1,2,3,4]. Such enzymes are:

- Pyruvate carboxylase (EC 6.4.1.1).
- Acetyl-CoA carboxylase (EC 6.4.1.2).
- Propionyl-CoA carboxylase (EC 6.4.1.3).
- Methylcrotonoyl-CoA carboxylase (EC 6.4.1.4).
- Geranoyl-CoA carboxylase (EC 6.4.1.5).
- Urea carboxylase (EC 6.3.4.6).
- Oxaloacetate decarboxylase (EC 4.1.1.3).
- Methylmalonyl-CoA decarboxylase (EC 4.1.1.41).
- Glutaconyl-CoA decarboxylase (EC 4.1.1.70).
- Methylmalonyl-CoA carboxyl-transferase (EC 2.1.3.1) (transcarboxylase).

Sequence data reveal that the region around the biocytin (biotin-lysine) residue is well conserved and can be used as a signature pattern.

Consensus pattern[GN]-[DEQTR]-x-[LIVMFY]-x(2)-[LIVM]-x-[AIV]-M-K-[LMAT]-x(3)-[LIVM]-x-[SAV] [K is the biotin attachment site] Note the domain around the biotin-binding lysine residue is evolutionary related to that around the lipoyl-binding lysine residue of 2-oxo acid dehydrogenase acyltransferases

[1] Knowles J.R. Annu. Rev. Biochem. 58:195-221(1989).

[2] Samols D., Thronton C.G., Murtif V.L., Kumar G.K., Haase F.C., Wood H.G. J. Biol. Chem. 263:6461-6464(1988).

[3] Goss N.H., Wood H.G. Meth. Enzymol. 107:261-278(1984).

[4] Shenoy B.C., Xie Y., Park V.L., Kumar G.K., Beegen H., Wood H.G., Samols D. J. Biol. Chem. 267:18407-18412(1992).

2-oxo acid dehydrogenases acyltransferase component lipoyl binding site

The 2-oxo acid dehydrogenase multienzyme complexes [1,2] from bacterial and eukaryotic sources catalyze the oxidative decarboxylation of 2-oxo acids to the corresponding acyl-CoA. The three members of this family of multienzyme complexes are:

- Pyruvate dehydrogenase complex (PDC).
- 2-oxoglutarate dehydrogenase complex (OGDC).
- Branched-chain 2-oxo acid dehydrogenase complex (BCOADC).

These three complexes share a common architecture: they are composed of multiple copies of three component enzymes - E1, E2 and E3. E1 is a thiamine pyrophosphate-dependent 2-oxo acid dehydrogenase, E2 a dihydrolipamide acyltransferase, and E3 an FAD-containing dihydrolipamide dehydrogenase. E2 acyltransferases have an essential cofactor, lipoic acid, which is covalently bound via a amide linkage to a lysine group. The E2 components of OGCD and BCOACD bind a single lipoyl group, while those of PDC bind either one (in yeast and in Bacillus), two (in mammals), or three (in Azotobacter and in Escherichia coli) lipoyl groups [3].

In addition to the E2 components of the three enzymatic complexes described above, a lipoic acid cofactor is also found in the following proteins:

- H-protein of the glycine cleavage system (GCS) [4]. GCS is a multienzyme complex of four protein components, which catalyzes the degradation of glycine. H protein shuttles the methylamine group of glycine from the P protein to the T protein. H-protein from either prokaryotes or eukaryotes binds a single lipoic group.
- Mammalian and yeast pyruvate dehydrogenase complexes differ from that of other sources, in that they contain, in small amounts, a protein of unknown function - designated protein X or component X. Its sequence is closely related to that of E2 subunits and seems to bind a lipoic group [5].
- Fast migrating protein (FMP) (gene acoC) from Alcaligenes eutrophus [6]. This protein is most probably a dihydrolipamide acyltransferase involved in acetoin metabolism.

A signature pattern was developed which allows the detection of the lipoyl-binding site.

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Consensus pattern[GN]-x(2)-[LIVF]-x(5)-[LIVFC]-x(2)-[LIVFA]-x(3)-K-[STAIV]-[STAVQDN]-x(2)-[LIVMFS]-x(5)-[GCN]-x-[LIVMFY] [K is the lipoyl-binding site] Note the domain around the lipoyl-binding lysine residue is evolutionary related to that around the biotin-binding lysine residue of biotin requiring enzymes

[1] Yeaman S.J. Biochem. J. 257:625-632(1989).

[2] Yeaman S.J. Trends Biochem. Sci. 11:293-296(1986).

[3] Russel G.C., Guest J.R. Biochim. Biophys. Acta 1076:225-232(1991).

[4] Fujiwara K., Okamura-Ikeda K., Motokawa Y. J. Biol. Chem. 261:8836-8841(1986).

[5] Behal R.H., Browning K.S., Hall T.B., Reed L.J. Proc. Natl. Acad. Sci. U.S.A. 86:8732-8736(1989).

[6] Priefert H., Hein S., Krueger N., Zeh K., Schmidt B., Steinbuechel A. J. Bacteriol. 173:4056-4071(1991).

70. C2 (C2 domain) Number of members: 295

Some isozymes of protein kinase C (PKC) [1,2] contain a domain, known as C2, of about 116 amino-acid residues which is located between the two copies of the C1 domain (that bind phorbol esters and diacylglycerol) (see <PDOC00379>) and the protein kinase catalytic domain (see <PDOC00100>). Regions with significant homology [3,E1] to the C2-domain have been found in the following proteins:

- PKC isoforms alpha, beta and gamma and Drosophila isoforms PKC1 and PKC2.
- PKC isoforms delta, epsilon and eta, Caenorhabditis elegans kin-13 and yeast PKC1 have a C2-like domain at the N-terminal extremity [4].
- Yeast cAMP dependent protein kinase SCH9 contains a C2-like domain.
- Mammalian phosphatidylinositol-specific phospholipase C (PI-PLC) (see <PDOC50007>) isoforms beta, gamma and delta as well as several non-mammalian PI-PLCs have a C2-like domain C-terminal of the catalytic domain.
- Mammalian and plants phosphatidylinositol-3-kinase have a C2-like domain in the central region of the 110 Kd catalytic subunit.
- Yeast phosphatidylserine-decarboxylase 2 (gene PSD2) contains a C2 domain in its central region.

- Cytosolic phospholipase D from plants and cytosolic phospholipase A2 have a C2-like domain at their N-terminus.
- Synaptotagmins (p65). This is a family of related synaptic vesicle proteins that bind acidic phospholipids and that may have a regulatory role in the membrane interactions during trafficking of synaptic vesicles at the active zone of the synapse. All isoforms of synaptotagmins have two copies of the C2 domain in their C-terminal region.
- Rabphilin-3A, a synaptic protein contains two C2 domains.
- Caenorhabditis elegans protein unc-13 whose function is not known. Unc-13 has a C2 domain in its central part and a C2-like domain at the C-terminus.
- rasGAP and the breakpoint cluster protein bcr have a C2-domain C-terminal of a PH-domain.
- Yeast protein BUD2 (or CLA2) has a C2-domain in the central region.
- Yeast protein RSP5 and human protein NEDD-4, both proteins also contain WW domains (see <PDOC50020>).
- Perforin (see <PDOC00251>) has a C2 domain at the C-terminus. It is the only extracellular protein known to contain a C2 domain.
- Yeast hypothetical protein YML072C has a C2 domain.
- Yeast hypothetical protein YNL087W has three C2 domains.
- Caenorhabditis elegans hypothetical protein F37A4.7 has two C2 domains.

The C2 domain is thought to be involved in calcium-dependent phospholipid binding [5]. Since domains related to the C2 domain are also found in proteins that do not bind calcium, other putative functions for the C2 domain like e.g. binding to inositol-1,3,4,5-tetraphosphate have been suggested [6]. Recently, the 3D structure of the first C2 domain of synaptotagmin has been reported [7], the domain forms an eight-stranded beta sandwich. The signature pattern that has been developed for the C2 domain is located in a conserved part of that domain, the connecting loop between beta strands 2 and 3. A profile has been developed for the C2 domain that covers the total domain.

-Consensus pattern: [ACG]-x(2)-L-x(2,3)-D-x(1,2)-[NGSTLIF]-[GTMR]-x-[STAP]-D-[PA]-[FY]

-Note: this documentation entry is linked to both a signature pattern and a profile. As the profile is much more sensitive than the pattern, you should use it if you have access to the necessary software tools to do so.

09555055.050402

[1]Medline: 96367095 Extending the C2 domain family: C2s in PKCs delta, epsilon, eta and theta, phospholipases, GAPs and perforin. Ponting CP, Parker PJ; Protein Sci 1996;5:162-166.

[1] Azzi A., Boscoboinik D., Hensey C. Eur. J. Biochem. 208:547-557(1992).

[2] Stabel S. Semin. Cancer Biol. 5:277-284(1994).

[3] Brose N., Hofmann K.O., Hata Y., Suedhof T.C. J. Biol. Chem. 270:25273-25280(1995).

[4] Sossin W.S., Schwartz J.H. Trends Biochem. Sci. 18:207-208(1993).

[5] Davletov B.A., Suedhof T.C. J. Biol. Chem. 268:26386-26390(1993).

[6] Fukuda M., Aruga J., Niinobe M., Aimoto S., Mikoshiba K. J. Biol. Chem. 269:29206-29211(1994).

[6] Sutton R.B., Davletov B.A., Berghuis A.M., Suedhof T.C., Sprang S.R. Cell 80:929-938(1995).

71. CAP (CAP protein) Number of members: 11

In budding and fission yeasts the CAP protein is a bifunctional protein whose N-terminal domain binds to adenylyl cyclase, thereby enabling that enzyme to be activated by upstream regulatory signals, such as Ras. The function of the C-terminal domain is less clear, but it is required for normal cellular morphology and growth control [1]. CAP is conserved in higher eukaryotic organisms where its function is not yet clear [2].

Structurally, CAP is a protein of 474 to 551 residues which consist of two domains separated by a proline-rich hinge. Two signature patterns, one corresponding to a conserved region in the N-terminal extremity and the other to a C-terminal region have been developed.

-Consensus pattern: [LIVM](2)-x-R-L-[DE]-x(4)-R-L-E

-Consensus pattern: D-[LIVMFY]-x-E-x-[PA]-x-P-E-Q-[LIVMFY]-K

[1] Kawamukai M., Gerst J., Field J., Riggs M., Rodgers L., Wigler M., Young D. Mol. Biol. Cell 3:167-180(1992).

[2] Yu G., Swiston J., Young D. J. Cell Sci. 107:1671-1678(1994).

72. CAP_GLY (CAP-Gly domain)

CAP stands for cytoskeleton-associated proteins. Swiss:P39937 may be a member but has not been included. It has a weak match to the family between residues 22-67. Number of members: 24

[1]Medline: 93242656. Sequence homologies between four cytoskeleton-associated proteins. Riehemann K, Sorg C; Trends Biochem Sci 1993;18:82-83.

It has been shown [1] that some cytoskeleton-associated proteins (CAP) share the presence of a conserved, glycine-rich domain of about 42 residues, called here CAP-Gly. Proteins known to contain this domain are listed below.

- Restin (also known as cytoplasmic linker protein-170 or CLIP-170), a 160 Kd protein associated with intermediate filaments and that links endocytic vesicles to microtubules. Restin contains two copies of the CAP-Gly domain.
- Vertebrate dynactin (150 Kd dynein-associated polypeptide; DAP) and Drosophila glued, a major component of activator I, a 20S polypeptide complex that stimulates dynein-mediated vesicle transport.
- Yeast protein BIK1 which seems to be required for the formation or stabilization of microtubules during mitosis and for spindle pole body fusion during conjugation.
- Yeast protein NIP100 (NIP80).
- Human protein CKAP1/TFCB, Schizosaccharomyces pombe protein alp11 and Caenorhabditis elegans hypothetical protein F53F4.3. These proteins contain a N-terminal ubiquitin domain (see <PDOC00271>) and a C-terminal CAP-Gly domain.
- Caenorhabditis elegans hypothetical protein M01A8.2.
- Yeast hypothetical protein YNL148c.

Structurally, these proteins are made of three distinct parts: an N-terminal section that is most probably globular and contains the CAP-Gly domain, a large central region predicted to be in an alpha-helical coiled-coil conformation and, finally, a short C-terminal globular domain. The signature for the CAP-Gly domain corresponds to the first 32 residues of the domain and includes five of the six conserved glycines.

-Consensus pattern: G-x(8,10)-[FYW]-x-G-[LIVM]-x-[LIVMFY]-x(4)-G-K-[NH]-x-G-[STAR]-x(2)-G-x(2)-[LY]-F

[1] Riehemann K., Sorg C. Trends Biochem. Sci. 18:82-83(1993).

73. (CBD 1)

Cellulose-binding domain, fungal type

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1].

Structurally, cellulases and xylanases generally consist of a catalytic domain joined to a cellulose-binding domain (CBD) by a short linker sequence rich in proline and/or hydroxy-amino acids.

The CBD of a number of fungal cellulases has been shown to consist of 36 amino acid residues. Enzymes known to contain such a domain are:

- Endoglucanase I (gene egl1) from Trichoderma reesei.
- Endoglucanase II (gene egl2) from Trichoderma reesei.
- Endoglucanase V (gene egl5) from Trichoderma reesei.
- Exocellobiohydrolase I (gene CBHI) from Humicola grisea, Neurospora crassa, Phanerochaete chrysosporium, Trichoderma reesei, and Trichoderma viride.
- Exocellobiohydrolase II (gene CBHII) from Trichoderma reesei.
- Exocellobiohydrolase 3 (gene cel3) from Agaricus bisporus
- Endoglucanases B, C2, F and K from Fusarium oxysporum.

The CBD domain is found either at the N-terminal (Cbh-II or egl2) or at the C-terminal extremity (Cbh-I, egl1 or egl5) of these enzymes. As it is shown in the following schematic representation, there are four conserved cysteines in this type of CBD domain, all involved in disulfide bonds.

```
+---------------+
|       +-----|--------+
        |      |   |     |
     xxxxxxxCxxxxxxxxxxxCxxxxxxCxxxxxxxxxxCx
        ****************************
```

'C': conserved cysteine involved in a disulfide bond.
'*': position of the pattern.

Such a domain has also been found in a putative polysaccharide binding protein from the red alga, Porphyra purpurea [2]. Structurally, this protein consists of four tandem repeats of the CBD domain.

Consensus patternC-G-G-x(4,7)-G-x(3)-C-x(5)-C-x(3,5)-[NHG]-x-[FYWM]- x(2)-Q-C [The four C's are involved in disulfide bonds] Sequences known to belong to this class detected by the pattern ALL.

[1] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).
[2] Liu Q., der Meer J.P., Reith M.E.

74. CBS domain. 3D Structure found as a subdomain in TIM barrel of inosine-. CBS domain web page. CBS domains are small intracellular modules mostly found in 2 or four copies within a protein. CBS domains are found in cystathionine-beta-synthase (CBS) where mutations lead to homocystinuria. Two CBS domains are found in inosine-monophosphate dehydrogenase from all species, however the CBS domains are not needed for activity. Two CBS domains are found in intracellular loops of several chloride channels. Mutations in this domain of Swiss:P35520 lead to homocystinuria.
Number of members: 414

[1]Medline: 97172695 The structure of a domain common to archaebacteria and the homocystinuria disease protein. Bateman A; Trends Biochem Sci 1997;22:12-13.
[2]Medline: 96279836 Structure and mechanism of inosine monophosphate dehydrogenase in complex with the immunosuppressant mycophenolic-acid. Sintchak MD, Fleming MA,

Futer O, Raybuck SA, Chambers SP, Caron PR, Murcko MA, Wilson KP; Cell 1996;85:921-930.

Discovery of CBS domain.

[3]Medline: 97259972 CBS domains in ClC chloride channels implicated in myotonia and nephrolithiasis (kidney stones). Ponting CP; J Mol Med 1997;75:160-163.

75. CDP-OH_P_transf (CDP-alcohol phosphatidyltransferase)

All of these members have the ability to catalyze the displacement of CMP from a CDP-alcohol by a second alcohol with formation of a phosphodiester bond and concomitant breaking of a phosphoride anhydride bond. Number of members: 32

A number of phosphatidyltransferases, which are all involved in phospholipid biosynthesis and that share the property of catalyzing the displacement of CMP from a CDP-alcohol by a second alcohol with formation of a phosphodiester bond and concomitant breaking of a phosphoride anhydride bond share a conserved sequence region [1,2]. These enzymes are:

- Ethanolaminephosphotransferase (EC 2.7.8.1) from yeast (gene EPT1).
- Diacylglycerol cholinephosphotransferase (EC 2.7.8.2) from yeast (gene CPT1).
- Phosphatidylglycerophosphate synthase (EC 2.7.8.5) (CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase) from bacteria (gene pgsA).
- Phosphatidylserine synthase (EC 2.7.8.8) (CDP-diacylglycerol--serine O-phosphatidyltransferase) from yeast (gene CHO1) and from Bacillus subtilis (gene pssA).
- Phosphatidylinositol synthase (EC 2.7.8.11) (CDP-diacylglycerol--inositol 3-phosphatidyltransferase) from yeast (gene PIS).

These enzymes are proteins of from 200 to 400 amino acid residues. The conserved region contains three aspartic acid residues and is located in the N-terminal section of the sequences.

-Consensus pattern: D-G-x(2)-A-R-x(8)-G-x(3)-D-x(3)-D

[1]Medline: 97075020 Two-dimensional 1H-NMR of transmembrane peptides from Escherichia coli phosphatidylglycerophosphate synthase in micelles. Morein S, Trouard TP, Hauksson JB, Rilfors L, Arvidson G, Lindblom G; Eur J Biochem 1996;241:489-497.

[1] Nikawa J.-I., Kodaki T., Yamashita S.

J. Biol. Chem. 262:4876-4881(1987).

[2] Hjelmstad R.H., Bell R.M.

J. Biol. Chem. 266:5094-5134(1991).

76. CHOD (Cholesterol oxidase) Members of the GMC oxidoreductase family. Number of members: 3

[1]Medline: 94032271. Crystal structure of cholesterol oxidase complexed with a steroid substrate: implications for flavin adenine dinucleotide dependent alcohol oxidases. Li J, Vrielink A, Brick P, Blow DM; Biochemistry 1993;32:11507-11515.

The following FAD flavoproteins oxidoreductases have been found [1,2] to be evolutionary related. These enzymes, which are called 'GMC oxidoreductases', are listed below.

- Glucose oxidase (EC 1.1.3.4) (GOX) from Aspergillus niger. Reaction catalyzed: glucose + oxygen -> delta-luconolactone + hydrogen peroxide.
- Methanol oxidase (EC 1.1.3.13) (MOX) from fungi. Reaction catalyzed: methanol + oxygen -> acetaldehyde + hydrogen peroxide.
- Choline dehydrogenase (EC 1.1.99.1) (CHD) from bacteria. Reaction catalyzed: choline + unknown acceptor -> betaine acetaldehyde + reduced acceptor.
- Glucose dehydrogenase (GLD) (EC 1.1.99.10) from Drosophila. Reaction catalyzed: glucose + unknown acceptor -> delta-gluconolactone + reduced acceptor.
- Cholesterol oxidase (CHOD) (EC 1.1.3.6) from Brevibacterium sterolicum and Streptomyces strain SA-COO. Reaction catalyzed: cholesterol + oxygen -> cholest-4-en-3-one + hydrogen peroxide.
- AlkJ [3], an alcohol dehydrogenase from Pseudomonas oleovorans, which converts aliphatic medium-chain-length alcohols into aldehydes. This family also includes a lyase:
- (R)-mandelonitrile lyase (EC 4.1.2.10) (hydroxynitrile lyase) from plants [4], an enzyme involved in cyanogenis, the release of hydrogen cyanide from injured tissues.

These enzymes are proteins of size ranging from 556 (CHD) to 664 (MOX) amino acid residues which share a number of regions of sequence similarities. One of these regions, located in the N-terminal section, corresponds to the FAD ADP- binding domain. The function of the other conserved domains is not yet known; two of these domains have been selected as signature patterns. The first one is located in the N-terminal section of these enzymes, about 50 residues after the ADP-binding domain, while the second one is located in the central section.

-Consensus pattern: [GA]-[RKN]-x-[LIV]-G(2)-[GST](2)-x-[LIVM]-N-x(3)-[FYWA]- x(2)-[PAG]-x(5)-[DNESH]
-Consensus pattern: [GS]-[PSTA]-x(2)-[ST]-P-x-[LIVM](2)-x(2)-S-G-[LIVM]-G

[1] Cavener D.R. J. Mol. Biol. 223:811-814(1992).
[2] Henikoff S., Henikoff J.G. Genomics 19:97-107(1994).
[3] van Beilen J.B., Eggink G., Enequist H., Bos R., Witholt B. Mol. Microbiol. 6:3121-3136(1992).
[4] Cheng I.P., Poulton J.E. Plant Cell Physiol. 34:1139-1143(1993).

77. CKS (Cyclin-dependent kinase regulatory subunit) Number of members: 11. Cyclin-dependent kinases (CDK) are protein kinases which associate with cyclins to regulate eukaryotic cell cycle progression. The most well known CDK is p34-cdc2 (CDC28 in yeast) which is required for entry into S-phase and mitosis. CDK's bind to a regulatory subunit which is essential for their biological function. This regulatory subunit is a small protein of 79 to 150 residues. In yeast (gene CKS1) and in fission yeast (gene suc1) a single isoform is known, while mammals have two highly related isoforms. It has been shown [1] that these CDK regulatory subunits assemble as an hexamer which then acts as a hub for the oligomerization of six CDK catalytic subunits. The sequence of CDK regulatory subunits are highly conserved therefore, the two most conserved regions have been used as signature patterns.

-Consensus pattern: Y-S-x-[KR]-Y-x-[DE](2)-x-[FY]-E-Y-R-H-V-x-[LV]-[PT]-[KRP]
-Consensus pattern: H-x-P-E-x-H-[IV]-L-L-F-[KR]

[1] Parge H.E., Arvai A.S., Murtari D.J., Reed S.I., Tainer J.A. Science 262:387-395(1993).

78. CK_II_beta (Casein kinase II regulatory subunit)

Number of members: 16. Casein kinase II (CK-2) [1] is an ubiquitous eukaryotic serine/threonine protein kinase which is found both in the cytoplasm and the nucleus and whose substrates are numerous. It generally phosphorylates Ser or Thr at the N-terminal of stretch of acidic residues (see <PDOC00006>). CK-2 exists as an heterotetramer composed of two catalytic subunits (alpha) and two regulatory subunits (beta). In most species there are two closely related isoforms of the catalytic subunit: alpha and alpha'. Some species, such as fungi and plants, express two forms of regulatory subunits: beta and beta'. The exact function of the regulatory subunit is not yet known. It is a highly conserved protein of about 25 Kd that contains, in its central section, a cysteine-rich motif that could be involved in binding a metal such as zinc [2]. This region has been used as a signature pattern.

-Consensus pattern: C-P-x-[LIVMY]-x-C-x(5)-[LI]-P-[LIVMC]-G-x(9)-V-[KR]-x(2)-C-P-x-C

[1] Allende J.E., Allende C.C. FASEB J. 9:313-323(1995).
[2] Reed J.C., Bidwai A.P., Glover C.V.C. J. Biol. Chem. 269:18192-18200(1994).

79. CLP_protease (Clp protease)

These proteins belong to family S14 in the classification of peptidases.

-!- The Clp protease has an active site catalytic triad. In E. coli Clp protease, ser-111, his-136 and asp-185 form the catalytic triad.

-!- Swiss:P48254 has lost all of these active site residues and is therefore inactive.

-!- Swiss:P42379 contains two large insertions, Swiss:P42380 contains one large insertion.

Number of members: 38

The endopeptidase Clp (EC 3.4.21.92) from Escherichia coli cleaves peptides in various proteins in a process that requires ATP hydrolysis [1,2]. Clp is a dimeric protein which consists of a proteolytic subunit (gene clpP) and either of two related ATP-binding regulatory subunits (genes clpA and clpX). ClpP is a serine protease which has a chymotrypsin-like activity. Its catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. Proteases highly similar to ClpP have been found to be encoded in the genome of the chloroplast of plants and seem to be also present in other eukaryotes. The sequences around two of the residues involved in the catalytic triad (a serine and a histidine) are highly conserved and can be used as signature patterns specific to that category of proteases.

-Consensus pattern: T-x(2)-[LIVMF]-G-x-A-[SAC]-S-[MSA]-[PAG]-[STA] [S is the active site residue]
-Consensus pattern: R-x(3)-[EAP]-x(3)-[LIVMFYT]-M-[LIVM]-H-Q-P [H is the active site residue]

[1]Medline: 98050920. The structure of ClpP at 2.3 angstroms resolution suggests a model for ATP-dependent proteolysis. Wang J, Hartling JA, Flanagan JM; Cell 1997;91:447-456.
[1] Maurizi M.R., Clark W.P., Kim S.-H., Gottesman S. J. Biol. Chem. 265:12546-12552(1990).
[2] Gottesman S., Maurizi M.R. Microbiol. Rev. 56:592-621(1992).
[3] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

80. CNG_membrane (Transmembrane region cyclic Nucleotide Gated Channel)
[1]Medline: 94224763. Cyclic nucleotide-gated channels: an expanding new family of ion channels. Yau KW; Proc Natl Acad Sci USA 1994;91:3481-3483.
This family is found to the N-terminus of the cNMP_binding. Number of members: 56.
Proteins that bind cyclic nucleotides (cAMP or cGMP) share a structural domain of about 120 residues [1-3]. The best studied of these proteins is the prokaryotic catabolite gene activator (also known as the cAMP receptor protein) (gene crp) where such a domain is known to be composed of three alpha-helices and a distinctive eight-stranded, antiparallel beta-barrel structure. Such a domain is known to exist in the following proteins:

- Prokaryotic catabolite gene activator protein (CAP).
- cAMP- and cGMP-dependent protein kinases (cAPK and cGPK). Both types of kinases contains two tandem copies of the cyclic nucleotide-binding domain. The cAPK's are composed of two different subunits: a catalytic chain and a regulatory chain which contains both copies of the domain. The cGPK's are single chain enzymes that include the two copies

of the domain in their N-terminal section. The nucleotide specificity of cAPK and cGPK is due to an amino acid in the conserved region of beta-barrel 7: a threonine that is invariant in cGPK is an alanine in most cAPK.

- Vertebrate cyclic nucleotide-gated ion-channels. Two such cations channels have been fully characterized. One is found in rod cells where it plays a role in visual signal transduction. It specifically binds to cGMP leading to an opening of the channel and thereby causing a depolarization of rod photoreceptors. In olfactory epithelium a similar, cAMP-binding, channel plays a role in odorant signal transduction. There are six invariant amino acids in this domain, three of which are glycine residues that are thought to be essential for maintenance of the structural integrity of the beta-barrel. Two signature patterns have been developed for this domain. The first pattern is located within beta-barrels and 3 and contains the first two conserved Gly. The second pattern is located within beta-barrels 6 and 7 and contains the third conserved Gly as well as the three other invariant residues.

-Consensus pattern: [LIVM]-[VIC]-x(2)-G-[DENQTA]-x-[GAC]-x(2)-[LIVMFY](4)-x(2)-G
-Consensus pattern: [LIVMF]-G-E-x-[GAS]-[LIVM]-x(5,11)-R-[STAQ]-A-x-[LIVMA]-x-[STACV]

[1] Weber I.T., Shabb J.B., Corbin J.D. Biochemistry 28:6122-6127(1989).
[2] Kaupp U.B. Trends Neurosci. 14:150-157(1991).
[3] Shabb J.B., Corbin J.D. J. Biol. Chem. 267:5723-5726(1992).

81. COX10_ctaB_cyoE (Cytochrome c oxidase assembly factor)
[1]Medline: 95191390
Biosynthesis and functional role of haem O and haem A
Mogi T, Saiki K, Anraku Y; Mol Microbiol 1994;14:391-398.
Cytochrome c oxidase is a multi subunit enzyme. The complexity of this enzyme requires assistance in building the complex.
This is carried out by the Cytochrome c oxidase assembly factor.
Number of members: 31

Cytochrome c oxidase is an oligomeric enzymatic complex which seems to require the aid of a number of proteins that either act as chaperonins to help the subunits of the enzyme to fold correctly, or assist in the assembly of the metal centers [1]. One of these subunits is known as COX10 in yeast and as ctaB [2] in aerobic prokaryotes. It is evolutionary related to cyoE protein from the Escherichia coli cytochrome O terminal oxidase complex.

These proteins probably contain [3] seven transmembrane segments. The most conserved region is located in a loop between the second and third of these segments and has been selected as a signature pattern.

-Consensus pattern: [ED]-x-D-x(2)-M-x-R-T-x(2)-R-x(4)-G

[1] Nobrega M.P., Nobrega F.G., Tzagoloff A.
J. Biol. Chem. 265:14220-14226(1990).
[2] Cao J., Hosler J., Shapleigh J., Revzin A., Ferguson-Miller S.
J. Biol. Chem. 267:24273-24278(1992).
[3] Chepuri V., Gennis R.B.
J. Biol. Chem. 265:12978-12986(1990).

09555325.061501

82. COX3 (Cytochrome c oxidase subunit III)

This family corresponds to chains c and p.

[1]Medline: 96216288

The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S; Science 1996;272:1136-1144.

Number of members: 224

83. COX5B (Cytochrome c oxidase subunit Vb)

[1]

Medline: 96216288

The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A.

Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S;

Science 1996;272:1136-1144.

This family consists of chains F and S

Number of members: 10

Cytochrome c oxidase (EC 1.9.3.1) [1] is an oligomeric enzymatic complex which is a component of the respiratory chain complex and is involved in the transfer of electrons from cytochrome c to oxygen. In eukaryotes this enzyme complex is located in the mitochondrial inner membrane; in aerobic prokaryotes it is found in the plasma membrane. In addition to the three large subunits that form the catalytic center of the enzyme complex there are, in eukaryotes, a variable number of small polypeptidic subunits. One of these subunits which is known as Vb in mammals, V in slime mold and IV in yeast, binds a zinc atom. The sequence of subunit Vb is well conserved and includes three conserved cysteines that are thought to coordinate the zinc ion [2]. Two of these cysteines are clustered in the C-terminal section of the subunit; this region has been selected as a signature pattern.

-Consensus pattern: [LIVM](2)-[FYW]-x(10)-C-x(2)-C-G-x(2)-[FY]-K-L [The two C's probably bind zinc]

[1] Capaldi R.A., Malatesta F., Darley-Usmar V.M.
Biochim. Biophys. Acta 726:135-148(1983).
[2] Rizzuto R., Sandona D., Brini M., Capaldi R.A., Bisson R.
Biochim. Biophys. Acta 1129:100-104(1991).

84. COesterase (Carboxylesterases)

Cholinesterase pages

The prints entry is specific to acetylcholinesterase

Number of members: 273

Higher eukaryotes have many distinct esterases. Among the different types are those which act on carboxylic esters (EC 3.1.1.-). Carboxyl-esterases have been classified into three categories (A, B and C) on the basis of differential patterns of inhibition by organophosphates. The sequence of a number of type-B carboxylesterases indicates [1,2,3] that the majority are evolutionary related. This family currently consists of the following proteins:

- Acetylcholinesterase (EC 3.1.1.7) (AChE) [E1] from vertebrates and from Drosophila.
- Mammalian cholinesterase II (butyryl cholinesterase) (EC 3.1.1.8). Acetylcholinesterase and cholinesterase II are closely related enzymes that hydrolyze choline esters [4].
- Mammalian liver microsomal carboxylesterases (EC 3.1.1.1).
- Drosophila esterase 6, produced in the anterior ejaculatory duct of the male insect reproductive system where it plays an important role in its reproductive biology.
- Drosophila esterase P.
- Culex pipiens (mosquito) esterases B1 and B2.
- Myzus persicae (peach-potato aphid) esterases E4 and FE4.
- Mammalian bile-salt-activated lipase (BAL) [5], a multifunctional lipase which catalyzes fat and vitamin absorption. It is activated by bile salts in infant intestine where it helps to digest milk fats.
- Insect juvenile hormone esterase (JH esterase) (EC 3.1.1.59).
- Lipases (EC 3.1.1.3) from the fungi Geotrichum candidum and Candida rugosa.
- Caenorhabditis gut esterase (gene ges-1).
- Duck fatty acyl-CoA hydrolase, medium chain (EC 3.1.2.14), an enzyme that may be associated with peroxisome proliferation and may play a role in the production of 3-hydroxy fatty acid diester pheromones.
- Membrane enclosed crystal proteins from slime mold. These proteins are, most probably esterases; the vesicles where they are found have therefore

been termed esterosomes.

So far two bacterial proteins have been found to belong to this family:

- Phenmedipham hydrolase (phenylcarbamate hydrolase), an Arthrobacter oxidans plasmid-encoded enzyme (gene pcd) that degrades the phenylcarbamate herbicides phenmedipham and desmedipham by hydrolyzing their central carbamate linkages.
- Para-nitrobenzyl esterase from Bacillus subtilis (gene pnbA).

The following proteins, while having lost their catalytic activity, contain a domain evolutionary related to that of carboxylesterases type-B:

- Thyroglobulin (TG), a glycoprotein specific to the thyroid gland, which is the precursor of the iodinated thyroid hormones thyroxine (T4) and triiodo thyronine (T3).
- Drosophila protein neuractin (gene nrt) which may mediate or modulate cell adhesion between embryonic cells during development.
- Drosophila protein glutactin (gene glt), whose function is not known.

09565596.061500

As is the case for lipases and serine proteases, the catalytic apparatus of esterases involves three residues (catalytic triad): a serine, a glutamate or aspartate and a histidine. The sequence around the active site serine is well conserved and can be used as a signature pattern. A conserved region located in the N-terminal section containing a cysteine involved in a disulfide bond has been selected as a second signature pattern.

-Consensus pattern: F-[GR]-G-x(4)-[LIVM]-x-[LIV]-x-G-x-S-[STAG]-G[S is the active site residue]

-Consensus pattern: [ED]-D-C-L-[YT]-[LIV]-[DNS]-[LIV]-[LIVFYW]-x-[PQR] [C is involved in a disulfide bond]

[1] Myers M., Richmond R.C., Oakeshott J.G. Mol. Biol. Evol. 5:113-119(1988).

[2] Krejci E., Duval N., Chatonnet A., Vincens P., Massoulie J. Proc. Natl. Acad. Sci. U.S.A. 88:6647-6651(1991).

[3] Cygler M., Schrag J.D., Sussman J.L., Harel M., Silman I. Gentry M.K., Doctor B.P. Protein Sci. 2:366-382(1993).

[4] Lockridge O. BioEssays 9:125-128(1988).

[5] Wang C.-S., Hartsuck J.A. Biochim. Biophys. Acta 1166:1-19(1993).

85. CPSase_L_chain (Carbamoyl-phosphate synthase (CPSase))

[1]

Medline: 94347758

Three-dimensional structure of the biotin carboxylase subunit. of acetyl-CoA carboxylase.

Waldrop GL, Rayment I, Holden HM;

Biochemistry 1994;33:10249-10256.

[1]

Medline: 90285162

Mammalian carbamyl phosphate synthetase (CPS). DNA sequence and evolution of the CPS domain of the Syrian hamster multifunctional protein CAD.

Simmer JP, Kelly RE, Rinker AG Jr, Scully JL, Evans DR;

Biol Chem 1990;265:10395-10402.

Carbamoyl-phosphate synthase catalyzes the ATP-dependent synthesis of carbamyl-phosphate from glutamine or ammonia and bicarbonate. This important enzyme initiates both the urea cycle and the biosynthesis of arginine and/or pyrimidines [2].

The carbamoyl-phosphate synthase (CPS) enzyme in prokaryotes is a heterodimer of a small and large chain. The small chain promotes the hydrolysis of glutamine to ammonia, which is used by the large chain to synthesize carbamoyl phosphate. See CPSase_sm_chain. The small chain has a GATase domain in the carboxyl terminus. See GATase.

Number of members: 181

Carbamoyl-phosphate synthase (CPSase) catalyzes the ATP-dependent synthesis of carbamyl-phosphate from glutamine (EC 6.3.5.5) or ammonia (EC 6.3.4.16) and bicarbonate [1]. This important enzyme initiates both the urea cycle and the biosynthesis of arginine and pyrimidines.

Glutamine-dependent CPSase (CPSase II) is involved in the biosynthesis of pyrimidines and purines. In bacteria such as Escherichia coli, a single enzyme is involved in both biosynthetic pathways while other bacteria have separate enzymes. The bacterial enzymes are formed of two subunits. A small chain (gene carA) that provides glutamine amidotransferase activity (GATase) necessary for removal of the ammonia group from glutamine, and a large chain (gene carB) that provides CPSase activity. Such a structure is also present in fungi for arginine biosynthesis (genes CPA1 and CPA2). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by a large multifunctional enzyme - called URA2 in yeast, rudimentary in Drosophila and CAD in mammals [2]. The CPSase domain is located between an N-terminal GATase domain and the C-terminal part which encompass the dihydroorotase and aspartate transcarbamylase activities.

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Ammonia-dependent CPSase (CPSase I) is involved in the urea cycle in ureolytic vertebrates; it is a monofunctional protein located in the mitochondrial matrix.

The CPSase domain is typically 120 Kd in size and has arisen from the duplication of an ancestral subdomain of about 500 amino acids. Each subdomain independently binds to ATP and it is suggested that the two homologous halves act separately, one to catalyze the phosphorylation of bicarbonate to carboxy phosphate and the other that of carbamate to carbamyl phosphate.

The CPSase subdomain is also present in a single copy in the biotin-dependent enzymes acetyl-CoA carboxylase (EC 6.4.1.2) (ACC), propionyl-CoA carboxylase (EC 6.4.1.3) (PCCase), pyruvate carboxylase (EC 6.4.1.1) (PC) and urea carboxylase (EC 6.3.4.6).

Two conserved regions which are probably important for binding ATP and/or catalytic activity have been selected as signatures for the subdomain.

-Consensus pattern: [FYV]-[PS]-[LIVMC]-[LIVMA]-[LIVM]-[KR]-[PSA]-[STA]-x(3)-[SG]-G-x-[AG]
-Consensus pattern: [LIVMF]-[LIMN]-E-[LIVMCA]-N-[PATLIVM]-[KR]-[LIVMSTAC]

[1] Simmer J.P., Kelly R.E., Rinker A.G. Jr., Scully J.L., Evans D.R.
J. Biol. Chem. 265:10395-10402(1990).
[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B.
BioEssays 15:157-164(1993).

86. CPSase_sm_chain (Carbamoyl-phosphate synthase small chain, CPSase domain) [1]

Medline: 90285162

Mammalian carbamyl phosphate synthetase (CPS). DNA sequence and evolution of the CPS domain of the Syrian hamster multifunctional protein CAD.

Simmer JP, Kelly RE, Rinker AG Jr, Scully JL, Evans DR;

Biol Chem 1990;265:10395-10402.

The carbamoyl-phosphate synthase domain is in the amino terminus of protein.

Carbamoyl-phosphate synthase catalyzes the ATP-dependent synthesis of carbamyl-phosphate from glutamine or ammonia and bicarbonate. This important enzyme initiates both the urea cycle and the biosynthesis of arginine and/or pyrimidines [1].

The carbamoyl-phosphate synthase (CPS) enzyme in prokaryotes is a heterodimer of a small and large chain. The small chain promotes the hydrolysis of glutamine to ammonia, which is used by the large chain to synthesize carbamoyl phosphate. See CPSase_L_chain.

The small chain has a GATase domain in the carboxyl terminus.

See GATase.

Number of members: 46

Carbamoyl-phosphate synthase (CPSase) catalyzes the ATP-dependent synthesis of carbamyl-phosphate from glutamine (EC 6.3.5.5) or ammonia (EC 6.3.4.16) and bicarbonate [1]. This important enzyme initiates both the urea cycle and the biosynthesis of arginine and pyrimidines.

Glutamine-dependent CPSase (CPSase II) is involved in the biosynthesis of pyrimidines and purines. In bacteria such as Escherichia coli, a single enzyme is involved in both biosynthetic pathways while other bacteria have separate enzymes. The bacterial enzymes are formed of two subunits. A small chain (gene carA) that provides glutamine amidotransferase activity (GATase) necessary for removal of the ammonia group from glutamine, and a large chain (gene carB) that provides CPSase activity. Such a structure is also present in fungi for arginine biosynthesis (genes CPA1 and CPA2). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by a large multifunctional enzyme - called URA2 in yeast, rudimentary in Drosophila and CAD in mammals [2]. The CPSase domain is located between an N-terminal GATase domain and the C-terminal part which encompass the dihydroorotase and aspartate transcarbamylase activities.

Ammonia-dependent CPSase (CPSase I) is involved in the urea cycle in ureolytic vertebrates; it is a monofunctional protein located in the mitochondrial matrix.

The CPSase domain is typically 120 Kd in size and has arisen from the duplication of an ancestral subdomain of about 500 amino acids. Each subdomain independently binds to ATP and it is suggested that the two homologous halves act separately, one to catalyze the phosphorylation of bicarbonate to carboxy phosphate and the other that of carbamate to carbamyl phosphate.

09554526.061502

The CPSase subdomain is also present in a single copy in the biotin-dependent enzymes acetyl-CoA carboxylase (EC 6.4.1.2) (ACC), propionyl-CoA carboxylase (EC 6.4.1.3) (PCCase), pyruvate carboxylase (EC 6.4.1.1) (PC) and urea carboxylase (EC 6.3.4.6).

Two conserved regions which are probably important for binding ATP and/or catalytic activity have been selected as signatures for the subdomain.

-Consensus pattern: [FYV]-[PS]-[LIVMC]-[LIVMA]-[LIVM]-[KR]-[PSA]-[STA]-x(3)-[SG]-G-x-[AG]

-Consensus pattern: [LIVMF]-[LIMN]-E-[LIVMCA]-N-[PATLIVM]-[KR]-[LIVMSTAC]

[1] Simmer J.P., Kelly R.E., Rinker A.G. Jr., Scully J.L., Evans D.R.
J. Biol. Chem. 265:10395-10402(1990).
[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B.
BioEssays 15:157-164(1993).

09595356.061500

87. CRAL_TRIO (CRAL/TRIO domain)

[1]
Medline: 98121119
Crystal structure of the Saccharomyces cerevisiae phosphatidyl-inositol-transfer protein.
Sha B, Phillips SE, Bankaitis VA, Luo M;
Nature 1998;391:506-510.

The original profile has been extended to include the carboxyl domain from the known structure of Sec14. Swiss:P10911 has not been included in the Pfam family because it does not appear to contain a complete structural domain.

Number of members: 39

88. CSD ('Cold-shock' DNA-binding domain)

[1]
Medline: 94255482
Crystal structure of CspA, the major cold shock protein of Escherichia coli.
Schindelin H, Jiang W, Inouye M, Heinemann U;
Proc Natl Acad Sci U S A 1994;91:5119-5123.
Number of members: 121

A conserved domain of about 70 amino acids has been found in prokaryotic and eukaryotic DNA-binding proteins [1,2,3,E1]. This domain, which is known as the 'cold-shock domain' (CSD) is present in the proteins listed below.

- Escherichia coli protein CS7.4 (gene cspA) which is induced in response to low temperature (cold-shock protein) and which binds to and stimulates the transcription of the CCAAT-containing promoters of the HN-S protein and of gyrA.
- Mammalian Y box binding protein 1 (YB1). A protein that binds to the CCAAT-containing Y box of mammalian HLA class II genes.
- Xenopus Y box binding proteins -1 and -2 (Y1 and Y2). Proteins that bind to the CCAAT-containing Y box of Xenopus hsp70 genes.
- Xenopus B box binding protein (YB3). YB3 binds the B box promoter element of genes transcribed by RNA polymerase III.
- Enhancer factor I subunit A (EFI-A) (dbpB). A protein that also bind to CCAAT-motif in various gene promoters.
- DbpA, a Human DNA-binding protein of unknown specificity.
- Bacillus subtilis cold-shock proteins cspB and cspC.
- Streptomyces clavuligerus protein SC 7.0.
- Escherichia coli proteins cspB, cspC, cspD, cspE and cspF.
- Unr, a mammalian gene encoded upstream of the N-ras gene. Unr contains nine repeats that are similar to the CSD domain. The function of Unr is not yet known but it could be a multivalent DNA-binding protein.

As a signature pattern for the CSD domain, its most conserved region which is located in its N-terminal section has been selected. It must be noted that the beginning of this region is highly similar [4] to the RNP-1 RNA-binding motif.

-Consensus pattern: [FY]-G-F-I-x(6,7)-[DER]-[LIVM]-F-x-H-x-[STKR]-x-[LIVMFY]

[1] Doniger J., Landsman D., Gonda M.A., Wistow G.
New Biol. 4:389-395(1992).
[2] Wistow G.
Nature 344:823-824(1990).
[3] Jones P.G., Inouye M.
Mol. Microbiol. 11:811-818(1994).
[4] Landsman D.
Nucleic Acids Res. 20:2861-2864(1992).

09525356.061500

89. CTF_NFI (CTF/NF-I family)
Number of members: 45

Nuclear factor I (NF-I) or CCAAT box-binding transcription factor (CTF) [1,2] (also known as TGGCA-binding proteins) are a family of vertebrate nuclear proteins which recognize and bind, as dimers, the palindromic DNA sequence 5'-TGGCANNNTGCCA-3'. CTF/NF-I binding sites are present in viral and cellular promoters and in the origin of DNA replication of Adenovirus type 2.

The CTF/NF-I proteins were first identified as nuclear factor I, a collection of proteins that activate the replication of several Adenovirus serotypes (together with NF-II and NF-III) [3]. The family of proteins was also identified as the CTF transcription factors, before the NFI and CTF families were found to be identical [4]. The CTF/NF-I proteins are individually capable of activating transcription and DNA replication. The CTF/NF-I family name has also been dubbed as NFI, NF-I or NF1.

In a given species, there are a large number of different CTF/NF-I proteins.

The multiplicity of CTF/NF-I is known to be generated both by alternative splicing and by the occurrence of four different genes. The known forms of NF-I genes have been classified as:

- The CTF-like factors subfamily (prototype form: CTF-1) [4]
- The NFI-X proteins.
- The NFI-A proteins.
- The NFI-B proteins.

So far, all CTF/NF-I family members appear to have similar transcription and replication activities.

CTF/NF-1 proteins contains 400 to 600 amino acids. The N-terminal 200 amino-acid sequence, almost perfectly conserved in all species and genes sequenced, mediates site-specific DNA recognition, protein dimerization and Adenovirus DNA replication. The C-terminal 100 amino acids contain the transcriptional activation domain. This activation domain is the target of gene expression regulatory pathways ellicited by growth factors and it interacts with basal transcription factors and with histone H3 [6].

A perfectly conserved, highly charged 12 residue peptide located in the N-terminal part of CTF/NF-I has been selected as a specific signature for this family of proteins.

-Consensus pattern: R-K-R-K-Y-F-K-K-H-E-K-R

[1] Mermod N., O'Neill E.A., Kelly T.J., Tjian R.
Cell 58:741-753(1989).
[2] Rupp R.A.W., Kruse U., Multhaup G., Goebel U., Beyreuther K., Sippel A.E.
Nucleic Acids Res. 18:2607-2616(1990).
[3] Nagata K., Guggenheimer R.A., Enomoto T., Lichy J.H., Hurwitz J.
Proc. Natl. Acad. Sci. U.S.A. 79:6438-6442(1982).
[4] Santoro C., Mermod N., Andrews P.C., Tjian R.

09595206.052601

Nature 334:2118-2224(1988).

[5] Gil G., Smith J.R., Goldstein J.L., Slaughter C.A., Orth K., Brown M.S., Osborne T.F.
Proc. Natl. Acad. Sci. U.S.A 85:8963-8967(1988).

[6] Alevizopoulos A., Dusserre Y., Tsai-Pflugfelder M., von der Weid T., Wahli W., Mermod N.
Genes Dev. 9:3051-3066(1995).

90. Calsequestrin (Calsequestrin)

Number of members: 13

09555326.060701

Calsequestrin is a moderate-affinity, high-capacity calcium-binding protein of cardiac and skeletal muscle [1], where it is located in the lumenal space of the sarcoplasmic reticulum terminal cisternae. Calsequestrin acts as a calcium buffer and plays an important role in the muscle excitation-contraction coupling. It is a highly acidic protein of about 400 amino acid residues that binds more than 40 moles of calcium per mole of protein. There are at least two different forms of calsequestrin: one which is expressed in cardiac muscles and another in skeletal muscles. Both forms have highly similar sequences.

Two signature sequences have been developed. The first corresponds to the N-terminus of the mature protein, the second is located just in front of the C-terminus of the protein which is composed of a highly acidic tail of variable length.

-Consensus pattern: [EQ]-[DE]-G-L-[DN]-F-P-x-Y-D-G-x-D-R-V

-Consensus pattern: [DE]-L-E-D-W-[LIVM]-E-D-V-L-x-G-x-[LIVM]-N-T-E-D-D-D

[1] Treves S., Vilsen B., Chiozzi P., Andersen J.P., Zorzato F.
Biochem. J. 283:767-772(1992).

91. Carboxyl_trans (Carboxyl transferase domain)

[1]

Medline: 93374821

Primary structure of the monomer of the 12S subunit of transcarboxylase as deduced from DNA and characterization of the product expressed in Escherichia coli.

Thornton CG, Kumar GK, Haase FC, Phillips NF, Woo SB, Park VM, Magner WJ, Shenoy BC, Wood HG, Samols D;

J Bacteriol 1993;175:5301-5308.

[2]

Medline: 93358891

Molecular evolution of biotin-dependent carboxylases.

Toh H, Kondo H, Tanabe T;

Eur J Biochem 1993;215:687-696.

All of the members in this family are biotin dependent carboxylases. The carboxyl transferase domain carries out the following reaction; transcarboxylation from biotin to an acceptor molecule. There are two recognised types of carboxyl transferase. One of them uses acyl-CoA and the other uses 2-oxo acid as the acceptor molecule of carbon dioxide. All of the members in this family utilise acyl-CoA as the acceptor molecule.

Number of members: 47

92. Chal_stil_synt (Chalcone and stilbene synthases)

Number of members: 146

Chalcone synthases (CHS) (EC 2.3.1.74) and stilbene synthases (STS) (formerly known as resveratrol synthases) are related plant enzymes [1]. CHS is an important enzyme in flavanoid biosynthesis and STS a key enzyme in stilbene-type phyloalexin biosynthesis. Both enzymes catalyze the addition of three molecules of malonyl-CoA to a starter CoA ester (a typical example is

09555350.051502

4-coumaroyl-CoA), producing either a chalcone (with CHS) or stilbene (with STS).

These enzymes are proteins of about 390 amino-acid residues. A conserved cysteine residue, located in the central section of these proteins, has been shown [2] to be essential for the catalytic activity of both enzymes and probably represents the binding site for the 4-coumaryl-CoA group. The region around this active site residue is well conserved and can be used as a signature pattern.

In addition to the plant enzymes, this family also includes Bacillus subtilis bcsA.

-Consensus pattern: R-[LIVMFYS]-x-[LIVM]-x-[QHG]-x-G-C-[FYNA]-[GA]-G-[GA]-[STAV]-x-[LIVMF]-[RA] [C is the active site residue]

[1] Schroeder J., Schroeder G.
Z. Naturforsch. 45C:1-8(1990).
[2] Lanz T., Tropf S., Marner F.-J., Schroeder J., Schroeder G.
J. Biol. Chem. 266:9971-9976(1991).

93. Chorismate_synt (Chorismate synthase)
Number of members: 19

Chorismate synthase (EC 4.6.1.4) catalyzes the last of the seven steps in the shikimate pathway which is used in prokaryotes, fungi and plants for the biosynthesis of aromatic amino acids. It catalyzes the 1,4-trans elimination of the phosphate group from 5-enolpyruvylshikimate-3-phosphate (EPSP) to form chorismate which can then be used in phenylalanine, tyrosine or tryptophan biosynthesis. Chorismate synthase requires the presence of a reduced flavin mononucleotide (FMNH2 or FADH2) for its activity.

09595555.051402

Chorismate synthase from various sources shows [1,2] a high degree of sequence conservation. It is a protein of about 360 to 400 amino-acid residues. Three signature patterns have been developed from conserved regions rich in basic residues (mostly arginines). The first is in the N-terminal section, the second is central and the third is C-terminal.

-Consensus pattern: G-E-S-H-[GC]-x(2)-[LIVM]-[GTV]-x-[LIVM](2)-[DE]-G-x-[PV]

-Consensus pattern: [GE]-R-[SA](2)-[SAG]-R-[EV]-[ST]-x(2)-[RH]-V-x(2)-G
-Consensus pattern: R-[SH]-D-[PSV]-[CSAV]-x(4)-[GAI]-x-[IVGSP]-[LIVM]-x-E-[STAH]-[LIVM]

[1] Schaller A., Schmid J., Leibinger U., Amrhein N.
J. Biol. Chem. 266:21434-21438(1991).
[2] Jones D.G.L., Reusser U., Braus G.H.
Mol. Microbiol. 5:2143-2152(1991).

09555555.051502

94. Clat_adaptor_s (Clathrin adaptor complex small chain)
Number of members: 21

Clathrin coated vesicles (CCV) mediate intracellular membrane traffic such as receptor mediated endocytosis. In addition to clathrin, the CCV are composed of a number of other components including oligomeric complexes which are known as adaptor or clathrin assembly proteins (AP) complexes [1]. The adaptor complexes are believed to interact with the cytoplasmic tails of membrane proteins, leading to their selection and concentration. In mammals two type of adaptor complexes are known: AP-1 which is associated with the Golgi complex and AP-2 which is associated with the plasma membrane. Both AP-1 and AP-2 are heterotetramers that consist of two large chains - the adaptins - (gamma and beta' in AP-1; alpha and beta in AP-2); a medium chain (AP47 in AP-1; AP50 in AP-2) and a small chain (AP19 in AP-1; AP17 in AP-2).

The small chains of AP-1 and AP-2 are evolutionary related proteins of about 18 Kd. Homologs of AP17 and AP19 have also been found in yeast (genes APS1/ YAP19 and APS2/YAP17) [2,3,4]. AP17 and AP19 are also related to the zeta-chain [5] of coatomer (zeta-cop), a cytosolic protein complex that reversibly associates with Golgi membranes to form vesicles that mediate biosynthetic protein transport from the endoplasmic reticulum, via the Golgi up to the trans Golgi network.

A conserved region in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: [LIVM](2)-Y-[KR]-x(4)-L-Y-F

[1] Pearse B.M., Robinson M.S.
Annu. Rev. Cell Biol. 6:151-171(1990).
[2] Kirchhausen T., Davis A.C., Frucht S., O'Brine Greco B., Payne G.S., Tubb B.
J. Biol. Chem. 266:11153-11157(1991).
[3] Nakai M., Takada T., Endo T.
Biochim. Biophys. Acta 1174:282-284(1993).
[4] Phan H.L., Finlay J.A., Chu D.S., Tan P.K., Kirchhausen T., Payne G.S.
EMBO J. 13:1706-1717(1994).
[5] Kuge O., Hara-Kuge S., Orci L., Ravazzola M., Amherdt M., Tanigawa G., Wieland F.T., Rothman J.E.
J. Cell Biol. 123:1727-1734(1993).

95. Clathrin_lg_ch (Clathrin light chain.)
Number of members: 8

Clathrin [1,2] is the major coat-forming protein that encloses vesicles such as coated pits and forms cell surface patches involved in membrane traffic within eukaryotic cells. The clathrin coats (called triskelions) are composed of three heavy chains (180 Kd) and three light chains (23 to 27 Kd).

The clathrin light chains [3], which may help to properly orient the assembly and disassembly of the clathrin coats, bind non-covalently to the heavy chain, they also bind calcium and interact with the hsc70 uncoating ATPase.

- In higher eukaryotes two genes code for distinct but related light chains: LC(a) and LC(b). Each of the two genes can yield, by tissue-specific alternative splicing, two separate forms which differ by the insertion of a sequence of respectively thirty or eighteen residues. There is, in the N-terminal part of the clathrin light chains a domain of twenty one amino acid residues which is perfectly conserved in LC(a) and LC(b).
- In yeast there is a single light chain (gene CLC1) whose sequence is only distantly related to that of higher eukaryotes.

Two signature patterns have been developed for clathrin light chains. The first pattern is a heptapeptide from the center of the conserved N-terminal region of eukaryotic light chains; the second pattern is derived from a positively charged region located in the C-terminal extremity of all known clathrin light chains.

-Consensus pattern: F-L-A-Q-Q-E-S

[1] Keen J.H.
Annu. Rev. Biochem. 59:415-438(1990).
[2] Brodsky F.M.
Science 242:1396-1402(1988).
[3] Brodsky F.M., Hill B.L., Acton S.L., Naethke I., Wong D.H., Ponnambalam S., Parham P.
Trends Biochem. Sci. 16:208-213(1991).

96. (Clathrin repeat) 7-fold repeat in Clathrin and VPS

Each repeat is about 140 amino acids long. The repeats occur in the arm region of the Clathrin heavy chain.

Number of members: 79

[1]

Medline: 92191269

Folding and trimerization of clathrin subunits at the triskelion hub.

Nathke IS, Heuser J, Lupas A, Stock J, Turck CW, Brodsky FM;

Cell 1992;68:899-910. [2]

Medline: 88097376

Clathrin heavy chain: molecular cloning and complete primary structure.

Kirchhausen T, Harrison SC, Chow EP, Mattaliano RJ, Ramachandran KL, Smart J, Brosius J;

Proc Natl Acad Sci U S A 1987;84:8805-8809.

09555526.062402

97. Collagen (Collagen triple helix repeat (20 copies))

[1] Medline: 94059583

New members of the collagen superfamily

Mayne R, Brewton RG;

Curr Opin Cell Biol 1993;5:883-890.

Scurvy is associated with collagens.

Members of this family belong to the collagen superfamily [1]. Collagens are generally extracellular structural proteins involved in formation of connective tissue structure. The alignment contains 20 copies of the G-X-Y repeat that forms a triple helix. The first position of the repeat is glycine, the second and third positions can be any residue but are frequently proline and hydroxyproline. Collagens are post translationally modified by proline hydoxylase to form the hydroxyproline residues. Defective hydroxylation is the cause of scurvy.

Some members of the collagen superfamily are not involved in connective tissue structure but share the same triple helical structure.

Number of members: 2125

98. Coprogen_oxidas (Coproporphyrinogen III oxidase)

Number of members: 12

Coproporphyrinogen III oxidase (EC 1.3.3.3) (coproporphyrinogenase) [1,2] catalyzes the oxidative decarboxylation of coproporphyrinogen III into protoporphyrinogen IX, a common step in the pathway for the biosynthesis of porphyrins such as heme, chlorophyll or cobalamin.

Coproporphyrinogen III oxidase is an enzyme that requires iron for its activity. A cysteine seems to be important for the catalytic mechanism [3]. Sequences from a variety of eukaryotic and prokaryotic sources show that this enzyme has been evolutionarily conserved. A highly conserved region in the central part of the sequence has been selected as a signature pattern. This region contains the only conserved cysteine and is rich in charged amino acids.

-Consensus pattern: K-x-W-C-x(2)-[FYH](3)-[LIVM]-x-H-R-x-E-x-R-G-[LIVM]-G-G-[LIVM]-F-F-D

[1] Xu K., Elliott T.
J. Bacteriol. 175:4990-4999(1993).

[2] Kohno H., Furukawa T., Yoshinaga T., Tokunaga R., Taketani S.
J. Biol. Chem. 268:21359-21363(1993).

[3] Camadro J.M., Chambon H., Jolles J., Labbe P.
Eur. J. Biochem. 156:579-587(1986).

[4] Xu K., Elliott T.
J. Bacteriol. 176:3196-3203(1994).

99. Corona_nucleoca (Coronavirus nucleocapsid protein)
[1]
Medline: 98087828
Identification of a specific interaction between the coronavirus mouse hepatitis virus A59 nucleocapsid protein and packaging signal.
Molenkamp R, Spaan WJ;
Virology 1997;239:78-86.
Number of members: 44

100. Cu-oxidase (Multicopper oxidase)
[1]
Medline: 90126844
The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin. Modelling and structural relationships.
Messerschmidt A, Huber R;
Eur J Biochem 1990;187:341-352.
Number of members: 150

Multicopper oxidases [1,2] are enzymes that possess three spectroscopically different copper centers. These centers are called: type 1 (or blue), type 2 (or normal) and type 3 (or coupled binuclear). The enzymes that belong to this family are:

- Laccase (EC 1.10.3.2) (urishiol oxidase), an enzyme found in fungi and plants, which oxidizes many different types of phenols and diamines.
- Ascorbate oxidase (EC 1.10.3.3), a higher plant enzyme.
- Ceruloplasmin (EC 1.16.3.1) (ferroxidase), a protein found in the serum of mammals and birds, which oxidizes a great variety of inorganic and organic substances. Structurally ceruloplasmin exhibits internal sequence homology, and seem to have evolved from the triplication of a copper-binding domain

similar to that found in laccase and ascorbate oxidase.

In addition to the above enzymes there are a number of proteins which, on the basis of sequence similarities, can be said to belong to this family. These proteins are:

- Copper resistance protein A (copA) from a plasmid in Pseudomonas syringae. This protein seems to be involved in the resistance of the microbial host to copper.
- Blood coagulation factor V (Fa V).
- Blood coagulation factor VIII (Fa VIII) [E1].
- Yeast FET3 [3], which is required for ferrous iron uptake.
- Yeast hypothetical protein YFL041w and SpAC1F7.08, the fission yeast homolog.

Factors V and VIII act as cofactors in blood coagulation and are structurally similar [4]. Their sequence consists of a triplicated A domain, a B domain and a duplicated C domain; in the following order: A-A-B-A-C-C. The A-type domain is related to the multicopper oxidases.

Two signature patterns have been developed for these proteins. Both patterns are derived from the same region, which in ascorbate oxidase, laccase, in the third domain of ceruloplasmin, and in copA, contains five residues that are known to be involved in the binding of copper centers. The first pattern does not make any assumption on the presence of copper-binding residues and thus can detect domains that have lost the ability to bind copper (such as those in Fa V and Fa VIII), while the second pattern is specific to copper-binding domains.

-Consensus pattern: G-x-[FYW]-x-[LIVMFYW]-x-[CST]-x(8)-G-[LM]-x(3)-[LIVMFYW]

-Consensus pattern: H-C-H-x(3)-H-x(3)-[AG]-[LM]

[The first two H's are copper type 3 binding residues]

[The C, the 3rd H, and L or M are copper type 1 ligands]

101. Cullin (Cullin family)
Number of members: 24

The following proteins are collectively termed cullins [1]:

- Caenorhabditis elegans cul-1 (or lin-19), a protein required for developmentally programmed transitions from the G1 phase of the cell cycle to the G0 phase or the apoptotic pathway.
- Caenorhabditis elegans cul-2, cul-3, cul-4 (F45E12.3), cul-5 (ZK856.1) and cul-6 (K08E7.7).
- Mammalian CUL1, CUL2, CUL3, CUL4A and CUL4B.
- Mammalian vasopressin-activated calcium-mobilizing receptor (VACM-1), a kidney-specific protein thought to form a cell surface receptor [2] but which does not have any structural hallmarks of a receptor.
- Drosophila lin19.
- Yeast CDC53 [3], which acts in concert with CDC4 and UBC3 (CDC34) to control the G1-to-S phase transition.
- Yeast hypothetical protein YGR003w.
- Fission yeast hypothetical protein SpAC24H6.03.

The cullins are hydrophilic proteins of 740 to 815 amino acids. The C-terminal extremity is the most conserved part of these proteins. A signature pattern has been developed from that region.

-Consensus pattern: [LIV]-K-x(2)-[LIV]-x(2)-L-I-[DEQ]-[KRHNQ]-x-Y-[LIVM]-x-R-x(6,7)-[FY]-x-Y-x-[SA]>

[1] Kipreos E.T., Lander L.E., Wing J.P., He W.W., Hedgecock E.M. Cell 85:829-839(1996).

[2] Burnatowska-Hledin M.A., Spielman W.S., Smith W.L., Shi P., Meyer J.M., Dewitt D.L.

Am. J. Physiol. 268:f1198-F1210(1995).

[3] Mathias N., Johnson S.L., Winey M., Adams A.E., Goetsch L., Pringle J.R., Byers B., Goebl M.G.
Mol. Cell. Biol. 16:6634-6643(1996).

102. (Cu_amine_oxid)

Copper amine oxidase signatures

Amine oxidases (AO) [1] are enzymes that catalyze the oxidation of a wide range of biogenic amines including many neurotransmitters, histamine and xenobiotic amines. There are two classes of amine oxidases: flavin-containing (EC 1.4.3.4) and copper-containing (EC 1.4.3.6).

Copper-containing AO is found in bacteria, fungi, plants and animals, it is an homodimeric enzyme that binds one copper ion per subunit as well as a 2,4,5- trihydroxyphenylalanine quinone (or topaquinone) (TPQ) cofactor. This cofactor is derived from a tyrosine residue.

Two signature patterns were derived for copper AO, the first one contains the tyrosine which give rises to the TPQ cofactor while the second one contains one of the three histidines that bind the copper atom [2].

Consensus pattern[LIVM]-[LIVMA]-[LIVMF]-x(4)-[ST]-x(2)-N-Y-[DE]-[YN] [The first Y gives rises to TPQ] Sequences known to belong to this class detected by the patternALL.

Consensus patternT-x-[GS]-x(2)-H-[LIVMF]-x(3)-E-[DE]-x-P [H is a copper ligand] Sequences known to belong to this class detected by the pattern ALL, except for lentil AO.

[1] Knowles P.F., Dooley D.M. (In) Metal ions in biological systems; Sigel H., Sigel A., Eds., 30:361- 403, Marcel Dekker, New-York, (1993).

[2] Parsons M.R., Convery M.A., Wilmot C.M., Yadav K.D.S., Blakeley V., Corner A.S., Phillips S.E.V., McPherson M.J., Knowles P.F. Structure 3:1171-1184(1995).

103. Cys-protease (Cysteine protease)

Number of members: 358

Eukaryotic thiol proteases (EC 3.4.22.-) [1] are a family of proteolytic enzymes which contain an active site cysteine. Catalysis proceeds through a thioester intermediate and is facilitated by a nearby histidine side chain; an asparagine completes the essential catalytic triad. The proteases which are currently known to belong to this family are listed below (references are only provided for recently determined sequences).

- Vertebrate lysosomal cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), L (EC 3.4.22.15), and S (EC 3.4.22.27) [2].
- Vertebrate lysosomal dipeptidyl peptidase I (EC 3.4.14.1) (also known as cathepsin C) [2].
- Vertebrate calpains (EC 3.4.22.17). Calpains are intracellular calcium-activated thiol protease that contain both a N-terminal catalytic domain and a C-terminal calcium-binding domain.
- Mammalian cathepsin K, which seems involved in osteoclastic bone resorption [3].
- Human cathepsin O [4].
- Bleomycin hydrolase. An enzyme that catalyzes the inactivation of the antitumor drug BLM (a glycopeptide).
- Plant enzymes: barley aleurain (EC 3.4.22.16), EP-B1/B4; kidney bean EP-C1, rice bean SH-EP; kiwi fruit actinidin (EC 3.4.22.14); papaya latex papain (EC 3.4.22.2), chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30), and proteinase IV (EC 3.4.22.25); pea turgor-responsive protein 15A; pineapple stem bromelain (EC 3.4.22.32); rape COT44; rice oryzain alpha, beta, and gamma; tomato low-temperature induced, Arabidopsis thaliana A494, RD19A and RD21A.
- House-dust mites allergens DerP1 and EurM1.
- Cathepsin B-like proteinases from the worms Caenorhabditis elegans (genes gcp-1, cpr-3, cpr-4, cpr-5 and cpr-6), Schistosoma mansoni (antigen SM31) and Japonica (antigen SJ31), Haemonchus contortus (genes AC-1 and AC-2), and Ostertagia ostertagi (CP-1 and CP-3).

- Slime mold cysteine proteinases CP1 and CP2.
- Cruzipain from Trypanosoma cruzi and brucei.
- Throphozoite cysteine proteinase (TCP) from various Plasmodium species.
- Proteases from Leishmania mexicana, Theileria annulata and Theileria parva.
- Baculoviruses cathepsin-like enzyme (v-cath).
- Drosophila small optic lobes protein (gene sol), a neuronal protein that contains a calpain-like domain.
- Yeast thiol protease BLH1/YCP1/LAP3.
- Caenorhabditis elegans hypothetical protein C06G4.2, a calpain-like protein.

Two bacterial peptidases are also part of this family:

- Aminopeptidase C from Lactococcus lactis (gene pepC) [5].
- Thiol protease tpr from Porphyromonas gingivalis.

Three other proteins are structurally related to this family, but may have lost their proteolytic activity.

- Soybean oil body protein P34. This protein has its active site cysteine replaced by a glycine.
- Rat testin, a sertoli cell secretory protein highly similar to cathepsin L but with the active site cysteine is replaced by a serine. Rat testin should not be confused with mouse testin which is a LIM-domain protein (see <PDOC00382>).
- Plasmodium falciparum serine-repeat protein (SERA), the major blood stage antigen. This protein of 111 Kd possesses a C-terminal thiol-protease-like domain [6], but the active site cysteine is replaced by a serine.

The sequences around the three active site residues are well conserved and can be used as signature patterns.

09555325.061502

-Consensus pattern: Q-x(3)-[GE]-x-C-[YW]-x(2)-[STAGC]-[STAGCV] [C is the active site residue]

-Consensus pattern: [LIVMGSTAN]-x-H-[GSACE]-[LIVM]-x-[LIVMAT](2)-G-x-[GSADNH] [H is the active site residue]

-Consensus pattern: [FYCH]-[WI]-[LIVT]-x-[KRQAG]-N-[ST]-W-x(3)-[FYW]-G-x(2)-G-[LFYW]-[LIVMFYG]-x-[LIVMF] [N is the active site residue]

[1] Dufour E. Biochimie 70:1335-1342(1988).
[2] Kirschke H., Barrett A.J., Rawlings N.D. Protein Prof. 2:1587-1643(1995).
[3] Shi G.-P., Chapman H.A., Bhairi S.M., Deleeuw C., Reddy V.Y., Weiss S.J. FEBS Lett. 357:129-134(1995).
[4] Velasco G., Ferrando A.A., Puente X.S., Sanchez L.M., Lopez-Otin C. J. Biol. Chem. 269:27136-27142(1994).
[5] Chapot-Chartier M.P., Nardi M., Chopin M.C., Chopin A., Gripon J.C. Appl. Environ. Microbiol. 59:330-333(1993).
[6] Higgins D.G., McConnell D.J., Sharp P.M. Nature 340:604-604(1989).
[7] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

104. Cys_Met_Meta_PP (Cys/Met metabolism PLP-dependent enzyme)

[1] Medline: 96428687
Crystal structure of the pyridoxal-5'-phosphate dependent cystathionine beta-lyase from Escherichia coli at 1.83 A.
Clausen T, Huber R, Laber B, Pohlenz HD, Messerschmidt A;
J Mol Biol 1996;262:202-224.

[1] Medline: 99059720
Crystal structure of Escherichia coli cystathionine gamma-synthase at 1.5 A resolution.
Clausen T, Huber R, Prade L, Wahl MC, Messerschmidt A;
EMBO J 1998;17:6827-6838.

Database Reference: SCOP; 1cs1; fa; [SCOP-USA][CATH-PDBSUM]

This family includes enzymes involved in cysteine and methionine metabolism. The following are members:

Cystathionine gamma-lyase,
Cystathionine gamma-synthase,
Cystathionine beta-lyase,
Methionine gamma-lyase,
OAH/OAS sulfhydrylase,
O-succinylhomoserine sulphhydrylase

All of these members participate is slightly different reactions.

All these enzymes use PLP (pyridoxal-5'-phosphate) as a cofactor.

Number of members: 52

09553562.061400

A number of pyridoxal-dependent enzymes involved in the metabolism of cysteine, homocysteine and methionine have been shown [1,2] to be evolutionary related. These are:

- Cystathionine gamma-lyase (EC 4.4.1.1) (gamma-cystathionase), which catalyzes the transformation of cystathionine into cysteine, oxobutanoate and ammonia. This is the final reaction in the transulfuration pathway that leads from methionine to cysteine in eukaryotes.
- Cystathionine gamma-synthase (EC 4.2.99.9), which catalyzes the conversion of cysteine and succinyl-homoserine into cystathionine and succinate: the first step in the biosynthesis of methionine from cysteine in bacteria (gene metB).
- Cystathionine beta-lyase (EC 4.4.1.8) (beta-cystathionase), which catalyzes the conversion of cystathionine into homocysteine, pyruvate and ammonia: the second step in the biosynthesis of methionine from cysteine in bacteria (gene metC).
- Methionine gamma-lyase (EC 4.4.1.11) (L-methioninase) which catalyzes the transformation of methionine into methanethiol, oxobutanoate and ammonia.
- OAH/OAS sulfhydrylase, which catalyzes the conversion of acetylhomoserine into homocysteine and that of acetylserine into cysteine (gene MET17 or MET25 in yeast).
- O-succinylhomoserine sulfhydrylase (EC 4.2.99.-).

- Yeast hypothetical protein YGL184c.
- Yeast hypothetical protein YHR112c.

These enzymes are proteins of about 400 amino-acid residues. The pyridoxal-P group is attached to a lysine residue located in the central section of these enzymes; the sequence around this residue is highly conserved and can be used as a signature pattern to detect this class of enzymes.

-Consensus pattern: [DQ]-[LIVMF]-x(3)-[STAGC]-[STAGCI]-T-K-[FYWQ]-[LIVMF]-x-G-[HQ]-[SGNH] [K is the pyridoxal-P attachment site]

[1] Ono B.I., Tanaka K., Naito K., Heike C., Shinoda S., Yamamoto S., Ohmori S., Oshima T., Toh-E A.
J. Bacteriol. 174:3339-3347(1992).

[2] Barton A.B., Kaback D.B., Clark M.W., Keng T., Ouellette B.F.F., Storms R.K., Zeng B., Zhong W.W., Fortin N., Delaney S., Bussey H.
Yeast 9:363-369(1993).

09565325.060700

105. Cyt_reductase

FAD/NAD-binding Cytochrome reductase

Number of members: 60

[1] Medline: 95111952

Crystal structure of the FAD-containing fragment of corn nitrate reductase at 2.5 A resolution: relationship to other flavoprotein reductases.

Lu G, Campbell WH, Schneider G, Lindqvist Y;

Structure 1994;2:809-821.

[2] Medline: 92084635

The sequence of squash NADH:nitrate reductase and its relationship to the sequences of other flavoprotein oxidoreductases. A family of flavoprotein pyridine nucleotide cytochrome reductases.

Hyde GE, Crawford NM, Campbell WH;
J Biol Chem 1991;266:23542-23547.

106. Cytidylyltrans
Phosphatidate cytidylyltransferase
Number of members: 21

Phosphatidate cytidylyltransferase (EC 2.7.7.41) [1,2,3] (also known as CDP-diacylglycerol synthase) (CDS) is the enzyme that catalyzes the synthesis of CDP-diacylglycerol from CTP and phosphatidate (PA). CDP-diacylglycerol is an important branch point intermediate in both prokaryotic and eukaryotic organisms. CDS is a membrane-bound enzyme. A conserved region located in the C-terminal part has been selected as a signature pattern.

-Consensus pattern: S-x-[LIVMF]-K-R-x(4)-K-D-x-[GSA]-x(2)-[LI]-[PG]-x-H-G-G-[LIVM]-x-D-R-[LIVMF]-D

[1] Sparrow C.P., Raetz C.R.H.
J. Biol. Chem. 260:12084-12091(1985).
[2] Shen H., Heacock P.N., Clancey C.J., Dowhan W.
J. Biol. Chem. 271:789-795(1996).
[3] Saito S., Goto K., Tonosaki A., Kondo H.
J. Biol. Chem. 272:9503-9509(1997).

107. (Cytidylyltransf) Cytidylyltransferase. This family includes: Cholinephosphate cytidylyltransferase. Glycerol-3-phosphate cytidylyltransferase.
Number of members: 64

[1] Medline: 10208837 CTP:Phosphocholine Cytidylyltransferase: Insights into Regulatory Mechanisms and Novel Functions. Clement JM, Kent C; Biochem Biophys Res Commun 1999;257:643-650.

09555326.061502

108. (cNMP binding) Cyclic nucleotide-binding domain signatures and profile

Proteins that bind cyclic nucleotides (cAMP or cGMP) share a structural domainof about 120 residues [1-3]. The best studied of these proteins is theprokaryotic catabolite gene activator (also known as the cAMP receptorprotein) (gene crp) where such a domain is known to be composed of threealpha-helices and a distinctive eight-stranded, antiparallel beta-barrelstructure. Such a domain is known to exist in the following proteins: - Prokaryotic catabolite gene activator protein (CAP). - cAMP- and cGMP-dependent protein kinases (cAPK and cGPK). Both types of kinases contains two tandem copies of the cyclic nucleotide-binding domain. The cAPK's are composed of two different subunits: a catalytic chain and a regulatory chain which contains both copies of the domain. The cGPK's are single chain enzymes that include the two copies of the domain in their N- terminal section. The nucleotide specificity of cAPK and cGPK is due to an amino acid in the conserved region of beta-barrel 7: a threonine that is invariant in cGPK is an alanine in most cAPK. - Vertebrate cyclic nucleotide-gated ion-channels. Two such cations channels have been fully characterized. One is found in rod cells where it plays a role in visual signal transduction. It specifically binds to cGMP leading to an opening of the channel and thereby causing a depolarization of rod photoreceptors. In olfactory epithelium a similar, cAMP-binding, channel plays a role in odorant signal transduction. There are six invariant amino acids in this domain, three of which are glycine residues that are thought to be essential for maintenance of the of the beta-barrel. Two signature patterns for this domain have been developed. The first pattern is located within beta-barrels 2 and 3 and contains the first two conserved Gly. The second pattern is located within beta-barrels 6 and 7 and contains the third conserved Gly as well as the three other invariant residues.-

First consensus pattern: [LIVM]-[VIC]-x(2)-G-[DENQTA]-x-[GAC]-x(2)-[LIVMFY](4)-x(2)-G

Second consensus pattern: [LIVMF]-G-E-x-[GAS]-[LIVM]-x(5,11)-R-[STAQ]-A-x-[LIVMA]-x- [STACV]-

[1] Weber I.T., Shabb J.B., Corbin J.D. Biochemistry 28:6122-6127(1989).

[2] Kaupp U.B. Trends Neurosci. 14:150-157(1991).

[3] Shabb J.B., Corbin J.D. J. Biol. Chem. 267:5723-5726(1992).

109. (cadherin)

Cadherins extracellular repeated domain signature

Cadherins [1,2] are a family of animal glycoproteins responsible for calcium-dependent cell-cell adhesion. Cadherins preferentially interact with themselves in a homophilic manner in connecting cells; thus acting as both receptor and ligand. A wide number of tissue-specific forms of cadherins are known:

- Epithelial (E-cadherin) (also known as uvomorulin or L-CAM) (CDH1).
- Neural (N-cadherin) (CDH2).
- Placental (P-cadherin) (CDH3).
- Retinal (R-cadherin) (CDH4).
- Vascular endothelial (VE-cadherin) (CDH5).
- Kidney (K-cadherin) (CDH6).
- Cadherin-8 (CDH8).
- Osteoblast (OB-cadherin) (CDH11).
- Brain (BR-cadherin) (CDH12).
- T-cadherin (truncated cadherin) (CDH13).
- Muscle (M-cadherin) (CDH14).
- Liver-intestine (LI-cadherin).
- EP-cadherin.

Structurally, cadherins are built of the following domains: a signal sequence, followed by a propeptide of about 130 residues, then an extracellular domain of around 600 residues, then a transmembrane region, and finally a C-terminal cytoplasmic domain of about 150 residues. The extracellular domain can be sub- divided into five parts: there are four repeats of about 110 residues followed by a region that contains four conserved cysteines. It is suggested that the calcium-binding region of cadherins is located in the extracellular repeats.

Cadherins are evolutionary related to the desmogleins which are component of intercellular desmosome junctions involved in the interaction of plaque proteins:

- Desmoglein 1 (desmosomal glycoprotein I).
- Desmoglein 2.
- Desmoglein 3 (Pemphigus vulgaris antigen).

The Drosophila fat protein [3] is a huge protein of over 5000 amino acids that contains 34 cadherin-like repeats in its extracellular domain.

The signature pattern that was developed for the repeated domain is located in it the C-terminal extremity which is its best conserved region. The pattern includes two conserved aspartic acid residues as well as two asparagines; these residues could be implicated in the binding of calcium.

Consensus pattern[LIV]-x-[LIV]-x-D-x-N-D-[NH]-x-P Sequences known to belong to this class detected by the pattern ALL. Note this pattern is found in the first, second, and fourth copies of the repeated domain. In the third copy there is a deletion of one residue after the second conserved Asp.

[1] Takeichi M. Annu. Rev. Biochem. 59:237-252(1990).
[2] Takeichi M. Trends Genet. 3:213-217(1987).
[3] Mahoney P.A., Weber U., Onofrechuk P., Biessmann H., Bryant P.J., Goodman C.S. Cell 67:853-868(1991).

110. Calreticulin family signatures

Calreticulin [1] (also known as calregulin, CRP55 or HACBP) is a high-capacitycalcium-binding protein which is present in most tissues and located at the periphery of the endoplasmic (ER) and the sarcoplamic reticulum (SR)membranes. It probably plays a role in the storage of calcium in the lumen ofthe ER and SR and it may well have other important functions. Structurally, calreticulin is a protein of about 400 amino acid residues consisting of three domains: a) An N-terminal, probably globular, domain of about 180 amino acid residues (N-domain); b) A central domain of about 70 residues (P-domain) which contains three repeats of an acidic 17 amino acid motif. This region binds calcium with a low-capacity, but a high-affinity; c) A C-terminal domain rich in acidic residues and in lysine (C-domain). This region binds calcium with a high-capacity but a low-affinity. Calreticulin is evolutionary related to the following proteins: - Onchocerca volvulus antigen RAL-1. RAL-1 is highly similar to calreticulin, but possesses a C-terminal domain rich in lysine and arginine and lacks acidic residues and is therefore not expected to bind calcium in that region. - Calnexin [2]. A calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins. - Calmegin [3] (or calnexin-T), a testis-specific calcium-binding protein highly similar to calnexin. Three signature patterns have been developed for this family of proteins. The first two patterns are based on conserved regions in the N-domain; the third pattern corresponds to positions 4 to 16 of the repeated motif in the P-domain.

Consensus pattern: [KRHN]-x-[DEQN]-[DEQNK]-x(3)-C-G-G-[AG]-[FY]-[LIVM]-[KN]-[LIVMFY](2)-

Consensus pattern: [LIVM](2)-F-G-P-D-x-C-[AG]-

Consensus pattern: [IV]-x-D-x-[DENST]-x(2)-K-P-[DEH]-D-W-[DEN]-

[1] Michalak M., Milner R.E., Burns K., Opas M. Biochem. J. 285:681-692(1992).

[2] Bergeron J.J.M., Brenner M.B., Thomas D.Y., Williams D.B. Trends Biochem. Sci. 19:124-128(1994).

[3] Watanabe D., Yamada K., Nishina Y., Tajima Y., Koshimizu U., Nagata A., Nishimune Y. J. Biol. Chem. 269:7744-7749(1994).

111. Eukaryotic-type carbonic anhydrases signature (carb_anhydrase)

Carbonic anhydrases (EC <u>4.2.1.1</u>) (CA) [1,2,3,4] are zinc metalloenzymes which catalyze the reversible hydration of carbon dioxide. Eight enzymatic and evolutionary related forms of carbonic anhydrase are currently known to exist in vertebrates: three cytosolic isozymes (CA-I, CA-II and CA-III); two membrane-bound forms (CA-IV and CA-VII); a mitochondrial form (CA-V); a secreted salivary form (CA-VI); and a yet uncharacterized isozyme [5].In the alga Chlamydomonas reinhardtii, two CA isozymes have been sequenced[6]. They are periplasmic glycoproteins evolutionary related to vertebrate CAs. Some bacteria, such as Neisseria gonorrhoeae [7] also have a eukaryotic-type CA.CAs contain a single zinc atom bound to three conserved histidine residues. As a signature for CAs, a pattern has been developed which includes one of these zinc-binding histidines. Protein D8 from Vaccinia and other poxviruses is related to CAs but has lost two of the zinc-binding histidines as well as many otherwise conserved residues. This is also true of the N-terminal extracellular domain of some receptor-type tyrosine-protein phosphatases (see <PDOC00323>).

Consensus pattern: S-E-[HN]-x-[LIVM]-x(4)-[FYH]-x(2)-E-[LIVMGA]-H-[LIVMFA](2) [The second H is a zinc ligand]-

Note: most prokaryotic CA's as well as plant chloroplast CA's belong to another, evolutionary distinct family of proteins (see <PDOC00586

[1] Deutsch H.F. Int. J. Biochem. 19:101-113(1987).

[2] Fernley R.T. Trends Biochem. Sci. 13:356-359(1988).

[3] Tashian R.E. BioEssays 10:186-192(1989).

[4] Edwards Y. Biochem. Soc. Trans. 18:171-175(1990).

[5] Skaggs L.A., Bergenhem N.C.H., Venta P.J., Tashian R.E. Gene 126:291-292(1993).

[6] Fujiwara S., Fukuzawa H., Tachiki A., Miyachi S. Proc. Natl. Acad. Sci. U.S.A. 87:9779-9783(1990).

[7] Huang S., Xue Y., Sauer-Eriksson E., Chirica L., Lindskog S.,Jonsson B.H. 2.3.CO;2-"J. Mol. Biol. 283:301-310(1998).

09535569.071902

112. Caseins alpha/beta signature

Caseins [1] are the major protein constituent of milk. Caseins can be classified into two families; the first consists of the kappa-caseins, and the second groups the alpha-s1, alpha-s2, and beta-caseins. The alpha/beta caseins are a rapidly diverging family of proteins. However two regions are conserved: a cluster of phosphorylated serine residues and the signal sequence. The signature pattern has been developed for this family of proteins based upon the last eight residues of the signal sequence.

Consensus pattern: C-L-[LV]-A-x-A-[LVF]-A –

[1] Holt C., Sawyer L. Protein Eng. 2:251-259(1988).

113. Catalase signatures

Catalase (EC 1.11.1.6) [1,2,3] is an enzyme, present in all aerobic cells,that decomposes hydrogen peroxide to molecular oxygen and water. Its main function is to protect cells from the toxic effects of hydrogen peroxide. In eukaryotic organisms and in some prokaryotes catalase is a molecule composed of four identical subunits. Each of the subunits binds one protoheme IX group. A conserved tyrosine serves as the heme proximal side ligand. The region around this residue has been used as a first signature pattern; it also includes a conserved arginine that participates in heme-binding. A conserved histidine has been shown to be important for the catalytic mechanism of the enzyme. The region around this residue has been selected as a second signature pattern.-

Consensus pattern: R-[LIVMFSTAN]-F-[GASTNP]-Y-x-D-[AST]-[QEH] [Y is the proximal heme-binding ligand]

Consensus pattern: [IF]-x-[RH]-x(4)-[EQ]-R-x(2)-H-x(2)-[GAS]-[GASTF]-[GAST] [H is an active site residue]

Note: some prokaryotic catalases belong to the peroxidase family (see <PDOC00394>).

[1] Murthy M.R.N., Reid T.J. III, Sicignano A., Tanaka N., Rossmann M.G. J. Mol. Biol. 152:465-499(1981).

[2] Melik-Adamyan W.R., Barynin V.V., Vagin A.A., Borisov V.V., Vainshtein B.K., Fita I., Murthy M.R.N., Rossmann M.G. J. Mol. Biol. 188:63-72(1986).

[3] von Ossowki I., Hausner G., Loewen P.C. J. Mol. Evol. 37:71-76(1993).

114. (chitin binding) Chitin recognition or binding domain signature

A conserved domain of 43 amino acids is found in several plant and fungal proteins that have a common binding specificity for oligosaccharides of N-acetylglucosamine [1]. This domain may be involved in the recognition or binding of chitin subunits. It has been found in the proteins listed below. - A number of non-leguminous plant lectins. The best characterized of these lectins are the three highly homologous wheat germ agglutinins (WGA-1, 2 and 3). WGA is an N-acetylglucosamine/N-acetylneuraminic acid binding lectin which structurally consists of a fourfold repetition of the 43 amino acid domain. The same type of structure is found in a barley root-specific lectin as well as a rice lectin. - Plants endochitinases (EC 3.2.1.14) from class IA (see <PDOC00620>). Endochitinases are enzymes that catalyze the hydrolysis of the beta-1,4 linkages of N-acetyl glucosamine polymers of chitin. Plant chitinases function as a defense against chitin containing fungal pathogens. Class IA chitinases generally contain one copy of the chitin-binding domain at their N-terminal extremity. An exception is agglutinin/chitinase [2] from the stinging nettle Urtica dioica which contains two copies of the domain. - Hevein [5], a wound-induced protein found in the latex of rubber trees. - Win1 and win2, two wound-induced proteins from potato. - Kluyveromyces lactis killer toxin alpha subunit [3]. The toxin encoded by the linear plasmid pGKL1 is composed of three subunits: alpha, beta, and gamma. The gamma subunit harbors toxin activity and inhibits growth of sensitive yeast strains in the G1 phase of the cell cycle; the alpha subunit, which is proteolytically processed from a larger precursor that also contains the beta subunit, is a chitinase (see <PDOC00839>). In chitinases, as well as in the potato wound-induced proteins, the 43-residuedomain directly follows the signal sequence and is therefore at the N-terminal of the mature protein; in the killer toxin alpha subunit it is located in the central section of the protein. The domain contains eight conserved cysteine residues which have all been shown, in WGA, to be involved in disulfide bonds. The topological arrangement of the four disulfide bonds is shown in the following figure: +-------------+ +----|------+ | | | | | xxCgxxxxxxxCxxxxCCsxxgxCgxxxxxCxxxCxxxxC | ******|************ | | | | +-----+ +--------------+'C': conserved cysteine involved in a disulfide bond.'*': position of the pattern.

-Consensus pattern: C-x(4,5)-C-C-S-x(2)-G-x-C-G-x(4)-[FYW]-C [The five C's are involved in disulfide bonds]

[1] Wright H.T., Sandrasegaram G., Wright C.S. J. Mol. Evol. 33:283-294(1991).
[2] Lerner D.R., Raikhel N.V. J. Biol. Chem. 267:11085-11091(1992).
[3] Butler A.R., O'Donnel R.W., Martin V.J., Gooday G.W., Stark M.J.R. Eur. J. Biochem. 199:483-488(1991).

115. (Chitinase 1) Chitinases family 19 signatures

Chitinases (EC 3.2.1.14) [1] are enzymes that catalyze the hydrolysis of thebeta-1,4-N-acetyl-D-glucosamine linkages in chitin polymers. From the viewpoint of sequence similarity chitinases belong to either family 18 or 19 in the classification of glycosyl hydrolases [2,E1]. Chitinases of family 19(also known as classes IA or I and IB or II) are enzymes from plants that function in the defense against fungal and insect pathogens by destroying their chitin-containing cell wall. Class IA/I and IB/II enzymes differ in the presence (IA/I) or absence (IB/II) of a N-terminal chitin-binding domain (seethe relevant entry <PDOC00025>). The catalytic domain of these enzymes consist of about 220 to 230 amino acid residues. Two highly conserved regions have been selected as signature patterns, the first one is located in the N-terminal section and contains one of the six cysteines which are conserved in most, if not all, of these chitinases and which is probably involved in a disulfide bond.

Consensus pattern: C-x(4,5)-F-Y-[ST]-x(3)-[FY]-[LIVMF]-x-A-x(3)-[YF]-x(2)-F- [GSA]
Consensus pattern: [LIVM]-[GSA]-F-x-[STAG](2)-[LIVMFY]-W-[FY]-W-[LIVM]

[1] Flach J., Pilet P.-E., Jolles P. Experientia 48:701-716(1992).
[2] Henrissat B. Biochem. J. 280:309-316(1991).

09555326 .061500

116. chloroa_b-bind
Chlorophyll A-B binding proteins. Number of members: 211

117. chromo
The 'chromo' (CHRromatin Organization MOdifier) domain [1 to 4] is a conserved region of about 60 amino acids which was originally found in Drosophila modifiers of variegation, which are proteins that modify the structure of chromatin to the condensed morphology of heterochromatin, a cytologically visible condition where gene expression is repressed. In protein Polycomb, the chromo domain has been shown to be important for chromatin targeting. Proteins that contains a chromo domain seem to fall into three classes:

a) Proteins which have a N-terminal chromo domain followed by a region which is related to but distinct from the chromo domain and which has been termed [3] the 'chromo shadow' domain.
b) Proteins with a single chromo domain.
c) Proteins with paired tandem chromo domains.

Currently, this domain has been found in the following proteins:

Class A.

- Drosophila heterochromatin protein Su(var)205 (HP1).
- Human heterochromatin protein HP1 alpha.
- Mammalian modifier 1 and modifier 2.
- Fission yeast swi6, a protein involved in the repression of the silent mating-type loci mat2 and mat3.

Class B.

- Drosophila protein Polycomb (Pc).
- Mammalian modifier 3, a homolog of Pc.
- Drosophila protein Su(var)3-9, a suppressor of position-effect variegation.
- Human Mi-2 autoantigen, characterisitic of dermatomyosis.
- Fungal retrotranposon polyproteins: 'skippy' from Fusarium oxysporum, 'grasshopper' and 'MAGGY' from Magnaporthe grisea and CfT-1 from Cladosporium fulvum.
- Fission yeast hypothetical protein SpAC18G6.02c.
- Caenorhabditis elegans hypothetical protein C29H12.5
- Caenorhabditis elegans hypothetical protein ZK1236.2.
- Caenorhabditis elegans hypothetical protein T09A5.8.

Class C.

- Mammalian DNA-binding/helicase proteins CHD-1 to CHD-4.
- Yeast protein CHD1.

The signature pattern for this domain corresponds to its best conserved section, which is located in its central part.

-Consensus pattern: [FYL]-x-[LIVMC]-[KR]-W-x-[GDNR]-[FYWLME]-x(5,6)-[ST]-W-[ESV]-[PSTDEN]-x(2,3)-[LIVMC]

09559570.041503

[1] Paro R. Trends Genet. 6:416-421(1990).

[2] Singh P.B., Miller J.R., Pearce J., Kothary R., Burton R.D., Paro R., James T.C., Gaunt S.J. Nucleic Acids Res. 19:789-794(1991).

[3] Aasland R., Stewart A.F. Nucleic Acids Res. 23:3168-3173(1995).

[4] Koonin E.V., Zhou S., Lucchesis J.C. Nucleic Acids Res. 23:4229-4233(1995).

118. citrate_synt

Citrate synthase (EC 4.1.3.7) (CS) is the tricarboxylic acid cycle enzyme that catalyzes the synthesis of citrate from oxaloacetate and acetyl-CoA in an aldol condensation. CS can directly form a carbon-carbon bond in the absence of metal ion cofactors.

In prokaryotes, citrate synthase is composed of six identical subunits. In eukaryotes, there are two isozymes of citrate synthase: one is found in the mitochondrial matrix, the second is cytoplasmic. Both seem to be dimers of identical chains.

There are a number of regions of sequence similarity between prokaryotic and eukaryotic citrate synthases. One of the best conserved contains a histidine which is one of three residues shown [1] to be involved in the catalytic mechanism of the vertebrate mitochondrial enzyme. This region has been used as a signature pattern.

-Consensus pattern: G-[FYA]-[GA]-H-x-[IV]-x(1,2)-[RKT]-x(2)-D-[PS]-R [H is an active site residue]

[1] Karpusas M., Branchaud B., Remington S.J. Biochemistry 29:2213-2219(1990).

119. clpA_B

Chaperonin clpA/B

CAUTION! This family is a subfamily of the AAA

09595345.052501

superfamily. The threshold has been set very high to stop overlaps with the AAA superfamily. This entry will be subsumed by AAA in the future.
Number of members: 39

A number of ATP-binding proteins that are are thought to protect cells from extreme stress by controlling the aggregation of denaturation of vital cellular structures have been shown [1,2] to be evolutionary related. These proteins are listed below.

- Escherichia coli clpA, which acts as the regulatory subunit of the ATP-dependent protease clp.
- Rhodopseudomonas blastica clpA homolog.
- Escherichia coli heat shock protein clpB and homologs in other bacteria.
- Bacillus subtilis protein mecB.
- Yeast heat shock protein 104 (gene HSP104), which is vital for tolerance to heat, ethanol and other stresses.
- Neurospora heat shock protein hsp98.
- Yeast mitochondrial heat shock protein 78 (gene HSP78) [3].
- CD4A and CD4b, two highly related tomato proteins that seem to be located in the chloroplast.
- Trypanosoma brucei protein clp.
- Porphyra purpurea chloroplast encoded clpC.

The size of these proteins range from 84 Kd (clpA) to slightly more than 100 Kd (HSP104). They all share two conserved regions of about 200 amino acids that each contains an ATP-binding site. In addition to the ATP-binding A and B motifs there are many parts in these two domains that are also conserved. Two of these regions have been selected as signature patterns. The first signature is located in the first domain, some ten residues to the C-terminal of the ATP-binding B motif. The second pattern is located in the second domain in-between the ATP-binding A and B motifs.

09595395.061400

-Consensus pattern: D-[AI]-[SGA]-N-[LIVMF](2)-K-[PT]-x-L-x(2)-G

-Consensus pattern: R-[LIVMFY]-D-x-S-E-[LIVMFY]-x-E-[KRQ]-x-[STA]-x-[STA]-[KR]-[LIVM]-x-G-[STA]

[1] Gottesman S., Squires C., Pichersky E., Carrington M., Hobbs M., Mattick J.S., Dalrymple B., Kuramitsu H., Shiroza T., Foster T., Clark W.P., Ross B., Squires C.L., Maurizi M.R. Proc. Natl. Acad. Sci. U.S.A. 87:3513-3517(1990).

[2] Parsell D.A., Sanchez Y., Stitzel J.D., Lindquist S. Nature 353:270-273(1991).

[3] Leonhardt S.A., Fearon K., Danese P.N., Mason T.L. Mol. Cell. Biol. 13:6304-6313(1993).

120. cofilin_ADF

Cofilin/tropomyosin-type actin-binding proteins

[1]

Medline: 97290449

Structure determination of yeast cofilin.

Fedorov AA, Lappalainen P, Fedorov EV, Drubin DG, Almo SC;

Nat Struct Biol 1997;4:366-369.

[2]

Medline: 97290450

Crystal structure of the actin-binding protein actophorin from Acanthamoeba.

Leonard SA, Gittis AG, Petrella EC, Pollard TD, Lattman EE;

Nat Struct Biol 1997;4:369-373.

[3]

Medline: 97420794

F-actin and G-actin binding are uncoupled by mutation of conserved tyrosine residues in maize actin depolymerizing factor.

Jiang CJ, Weeds AG, Khan S, Hussey PJ;

Proc Natl Acad Sci U S A 1997;94:9973-9978.

[4]

Medline: 97357155

Cofilin promotes rapid actin filament turnover in vivo.

Lappalainen P, Drubin DG;

Nature 1997;388:78-82.

Severs actin filaments and binds to actin monomers.

Number of members: 44

Actin-depolymerizing proteins sever actin filaments (F-actin) and/or bind to actin monomers, or G-actin, thus preventing actin-polymerization by sequestering the monomers. The following proteins are evolutionary related and belong to a family of low molecular weight (137 to 166 residues) actin-depolymerizing proteins [1,2,3,4]:

- Cofilin from vertebrates, slime mold and yeast. Cofilin binds to F-actin and acts as a pH-dependent actin-depolymerizing protein.
- Destrin from vertebrates. Destrin binds to G-actin in a pH-independent manner and prevents polymerization.
- Caenorhabditis elegans unc-60.
- Acanthamoeba castellanii actophorin.
- Plants actin depolymerizing factor (ADF).

The most conserved region of these proteins is a twenty amino-acid segment that ends some 30 residues from their C-terminal extremity. This segment has been shown [5] to be important for actin-binding.

-Consensus pattern: P-[DE]-x-[SA]-x-[LIVMT]-[KR]-x-[KR]-M-[LIVM]-[YA]-[STA](3)-x(3)-[LIVMF]-[KR]

[1] Hawkins M., Pope B., MacIver S.K., Weeds A.G. Biochemistry 32:9985-9993(1993).
[2] Iida K., Moriyama K., Matsumoto S., Kawasaki H., Nishida E., Yahara I. Gene 124:115-120(1993).
[3] Quirk S., MacIver S.K., Ampe C., Doberstein S.K., Kaiser D.A., van Damme J., Vandekerckhove J., Pollard T.D. Biochemistry 32:8525-8533(1993).

[4] McKim K.S., Matheson C., Marra M.A., Wakarchuk M.F., Baillie D.L. Mol. Gen. Genet. 242:346-357(1994).

[5] Moriyama K., Yonezawa N., Sakai H., Yahara I., Nishida E. J. Biol. Chem. 267:7240-7244(1992).

121. (Complex 24kd) Respiratory-chain NADH dehydrogenase 24 Kd subunit signature Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complexI or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist inthe chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase).Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 24 Kd (in mammals), which is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind a2Fe-2S iron-sulfur cluster. The 24 Kd subunit is nuclear encoded, as aprecursor form with a transit peptide in mammals, and in Neurospora crassa.The 24 Kd subunit is highly similar to [3,4]: - Subunit E of Escherichia coli NADH-ubiquinone oxidoreductase (gene nuoE). - Subunit NQO2 of Paracoccus denitrificans NADH-ubiquinone oxidoreductase. A highly conserved region, located in the central section of this subunit containing two conserved cysteines that are probably involved in the binding of the 2Fe-2S center has been selected as a signature pattern.

-Consensus pattern: D-x(2)-F-[ST]-x(5)-C-L-G-x-C-x(2) [GA]-P [The two C's are putative 2Fe-2S ligands]

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).

[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).

[3] Fearnley I.M., Walker J.E. Biochim. Biophys. Acta 1140:105-134(1992).

[4] Weidner U., Geier S., Ptock A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-122(1993).

122. copper-bind

Copper binding proteins, plastocyanin/azurin family

Number of members: 70

Blue or 'type-1' copper proteins are small proteins which bind a single copper atom and which are characterized by an intense electronic absorption band near 600 nm [1,2]. The most well known members of this class of proteins are the plant chloroplastic plastocyanins, which exchange electrons with cytochrome c6, and the distantly related bacterial azurins, which exchange electrons with cytochrome c551. This family of proteins also includes all the proteins listed below (references are only provided for recently determined sequences).

- Amicyanin from bacteria such as Methylobacterium extorquens or Thiobacillus versutus that can grow on methylamine. Amicyanin appears to be an electron receptor for methylamine dehydrogenase.
- Auracyanins A and B from Chloroflexus aurantiacus [3]. These proteins can donate electrons to cytochrome c-554.
- Blue copper protein from Alcaligenes faecalis.
- Cupredoxin (CPC) from cucumber peelings [4].
- Cusacyanin (basic blue protein; plantacyanin, CBP) from cucumber.
- Halocyanin from Natrobacterium pharaonis [5], a membrane associated copper-binding protein.
- Pseudoazurin from Pseudomonas.
- Rusticyanin from Thiobacillus ferrooxidans. Rusticyanin is an electron carrier from cytochrome c-552 to the a-type oxidase [6].
- Stellacyanin from the Japanese lacquer tree.
- Umecyanin from horseradish roots.

- Allergen Ra3 from ragweed. This pollen protein is evolutionary related to the above proteins, but seems to have lost the ability to bind copper.

Although there is an appreciable amount of divergence in the sequence of all these proteins, the copper ligand sites are conserved and a pattern which includes two of the ligands (a cysteine and a histidine) has been developed.

-Consensus pattern: [GA]-x(0,2)-[YSA]-x(0,1)-[VFY]-x-C-x(1,2)-[PG]-x(0,1)-H-x(2,4)-[MQ] [C and H are copper ligands]

[1] Garret T.P.J., Clingeleffer D.J., Guss J.M., Rogers S.J., Freeman H.C. J. Biol. Chem. 259:2822-2825(1984).

[2] Ryden L.G., Hunt L.T. J. Mol. Evol. 36:41-66(1993).

[3] McManus J.D., Brune D.C., Han J., Sanders-Loehr J., Meyer T.E., Cusanovich M.A., Tollin G., Blankenship R.E. J. Biol. Chem. 267:6531-6540(1992).

[4] Mann K., Schaefer W., Thoenes U., Messerschmidt A., Mehrabian Z., Nalbandyan R. FEBS Lett. 314:220-223(1992).

[5] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

[6] Yano T., Fukumori Y., Yamanaka T. FEBS Lett. 288:159-162(1991).

123. Chaperonins cpn10 signature

Chaperonins [1,2] are proteins involved in the folding of proteins or the assembly of oligomeric protein complexes. They seem to assist other polypeptides in maintaining or assuming conformations which permit their correct assembly into oligomeric structures. They are found in abundance in prokaryotes, chloroplasts and mitochondria. Chaperonins form oligomeric complexes and are composed of two different types of subunits: a 60 Kd protein, known as cpn60 (groEL in bacteria) and a 10 Kd protein, known ascpn10 (groES in bacteria).The cpn10 protein binds to cpn60 in the presence of MgATP and suppresses the ATPase activity of the latter. Cpn10 is a protein of about 100 amino acid residues whose sequence is well conserved in bacteria, vertebrate mitochondriaand plants chloroplast [3,4]. Cpn10 assembles as an heptamer that forms a dome[5]. As a signature pattern for cpn10 , a region located in the N-terminal section of the protein was selected.

Consensus pattern: [LIVMFY]-x-P-[ILT]-x-[DEN]-[KR]-[LIVMFA](3)-[KREQ]-x(8,9)-[SG]-x-[LIVMFY](3)-

Note: this pattern is found twice in the plant chloroplast protein which consist of the tandem repeat of a cpn10 domain

[1] Ellis R.J., van der Vies S.M. Annu. Rev. Biochem. 60:321-347(1991).

[2] Zeilsta-Ryalls J., Fayet O., Georgopoulos C. Annu. Rev. Microbiol. 45:301-325(1991).

[3] Hartman D.J., Hoogenraad N.J., Condron R., Hoj P.B. Proc. Natl. Acad. Sci. U.S.A. 89:3394-3398(1992).

[4] Bertsch U., Soll J., Seetharam R., Viitanen P.V. Proc. Natl. Acad. Sci. U.S.A. 89:8696-8700(1992).

[5] Hunt J.F., Weaver A.J., Landry S.J., Gierasch L., Deisenhofer J. Nature 379:37-45(1996).

124. Chaperonins cpn60 signature (cpn60_TCP1)

Chaperonins [1,2] are proteins involved in the folding of proteins or the assembly of oligomeric protein complexes. Their role seems to be to assist other polypeptides to maintain or assume conformations which permit their correct assembly into oligomeric structures. They are found in abundance in prokaryotes, chloroplasts and mitochondria. Chaperonins form oligomeric complexes and are composed of two different types of subunits: a 60 Kd protein, known as cpn60 (groEL in bacteria) and a 10 Kd protein, known as cpn10 (groES in bacteria).The cpn60 protein shows weak ATPase activity and is a highly conserved protein of about 550 to 580 amino acid residues which has been described by different names in different species: - Escherichia coli groEL protein, which is essential for the growth of the bacteria and the assembly of several bacteriophages. - Cyanobacterial groEL analogues. - Mycobacterium tuberculosis and leprae 65 Kd antigen, Coxiella burnetti heat shock protein B (gene htpB), Rickettsia tsutsugamushi major antigen 58, and Chlamydial 57 Kd hypersensitivity antigen (gene hypB). - Chloroplast RuBisCO subunit binding-protein alpha and beta chains, which bind ribulose bisphosphate carboxylase small and large subunits and are implicated in the assembly of the enzyme oligomer. - Mammalian mitochondrial matrix protein P1 (mitonin or P60). - Yeast HSP60 protein, a mitochondrial assembly factor. As a signature pattern for these proteins, a rather well-conserved region of twelve residues, located in the last third of the cpn60sequence was chosen.

Consensus pattern: A-[AS]-x-[DEQ]-E-x(4)-G-G-[GA]-

[1] Ellis R.J., van der Vies S.M. Annu. Rev. Biochem. 60:321-347(1991).

[2] Zeilsta-Ryalls J., Fayet O., Georgopoulos C. Annu. Rev. Microbiol. 45:301-325(1991).

Chaperonins TCP-1 signatures (cpn60_TCP1)

The TCP-1 protein [1,2] (Tailless Complex Polypeptide 1) was first identified in mice where it is especially abundant in testis but present in all cell types. It has since been found and characterized in many other mammalian species, in Drosophila and in yeast. TCP-1 is a highly conserved protein of about 60 Kd (556 to 560 residues) which participates in a hetero-oligomeric900 Kd double-torus shaped particle [3] with 6 to 8 other different subunits. These subunits, the chaperonin containing TCP-1 (CCT) subunit beta, gamma,delta, epsilon, zeta and eta are evolutionary related to TCP-1 itself [4,5].The CCT is known to act as a molecular chaperone for tubulin, actin and probably some other proteins. The CCT subunits are highly related to archebacterial counterparts: - TF55 and TF56 [6], a molecular chaperone from Sulfolobus shibatae. TF55 has ATPase activity, is known to bind unfolded polypeptides and forms a oligomeric complex of two stacked nine-membered rings. - Thermosome [7], from Thermoplasma acidophilum. The thermosome is composed of two subunits (alpha and beta) and also seems to be a chaperone with ATPase activity. It forms an oligomeric complex of eight-membered rings. The TCP-1 family of proteins are weakly, but significantly [8], related to thecpn60/groEL chaperonin family (see <PDOC00268>).As signature patterns of this family of chaperonins, three conserved regions located in the N-terminal domain were chosen.

Consensus pattern: [RKEL]-[ST]-x-[LMFY]-G-P-x-[GSA]-x-x-K-[LIVMF](2)-
Consensus pattern: [LIVM]-[TS]-[NK]-D-[GA]-[AVNHK]-[TAV]-[LIVM](2)-x(2)-[LIVM]-x-[LIVM]-x-[SNH]-[PQH]-
Consensus pattern: Q-[DEK]-x-x-[LIVMGTA]-[GA]-D-G-T-

[1] Ellis J. Nature 358:191-192(1992).
[2] Nelson R.J., Craig E.A. Curr. Biol. 2:487-489(1992).
[3] Lewis V.A., Hynes G.M., Zheng D., Saibil H., Willison K.R. Nature 358:249-252(1992).
[4] Kubota H., Hynes G., Carne A., Ashworth A., Willison K.R. Curr. Biol. 4:89-99(1994)
[5] Kim S., Willison K.R., Horwich A.L. Trends Biochem. Sci. 20:543-548(1994).
[6] Trent J.D., Nimmesgern E., Wall J.S., Hartl F.U., Horwich A.L. Nature 354:490-493(1991).

[7] Waldmann T., Lupas A., Kellermann J., Peters J., Baumeister W. Biol. Chem. Hoppe-Seyler 376:119-126(1995).

[8] Hemmingsen S.M. Nature 357:650-650(1992).

125. cyclin (Cyclins)

The cyclins include an internal duplication, which is related to that found in TFIIB and the RB protein.

[1]

Medline: 94203808

Evidence for a protein domain superfamily shared by the cyclins, TFIIB and RB/p107.

Gibson TJ, Thompson JD, Blocker A, Kouzarides T;

Nucleic Acids Res 1994;22:946-952.

[2]

Medline: 96164440

The crystal structure of cyclin A

Brown NR, Noble MEM, Endicott JA, Garman EF, Wakatsuki S, Mitchell E, Rasmussen B, Hunt T, Johnson LN;

Structure. 1995;3:1235-1247.

Complex of cyclin and cyclin dependant kinase.

[3]

Medline: 96313126

Structural basis of cyclin-dependant kinase activation by phosphorylation.

Russo AA, Jeffrey PD, Pavletich NP;

Nat Struct Biol. 1996;3:696-700.

Cyclins regulate cyclin dependant kinases (CDKs).

The most divergent prosite members have been included. Swiss:P22674 the Uracil-DNA glycosylase 2 is the highest noise and may be related but has not been included.

Number of members: 189

09555365.051501

Cyclins [1,2,3] are eukaryotic proteins which play an active role in controlling nuclear cell division cycles. Cyclins, together with the p34 (cdc2) or cdk2 kinases, form the Maturation Promoting Factor (MPF). There are two main groups of cyclins:

- G2/M cyclins, essential for the control of the cell cycle at the G2/M (mitosis) transition. G2/M cyclins accumulate steadily during G2 and are abruptly destroyed as cells exit from mitosis (at the end of the M-phase).
- G1/S cyclins, essential for the control of the cell cycle at the G1/S (start) transition.

In most species, there are multiple forms of G1 and G2 cyclins. For example, in vertebrates, there are two G2 cyclins, A and B, and at least three G1 cyclins, C, D, and E.

A cyclin homolog has also been found in herpesvirus saimiri [4].

The best conserved region is in the central part of the cyclins' sequences, known as the 'cyclin-box'. From this, a 32 residue pattern has been derived.

-Consensus pattern: R-x(2)-[LIVMSA]-x(2)-[FYWS]-[LIVM]-x(8)-[LIVMFC]-x(4)-[LIVMFYA]-x(2)-[STAGC]-[LIVMFYQ]-x-[LIVMFYC]-[LIVMFY]-D-[RKH]-[LIVMFYW]

[1] Nurse P. Nature 344:503-508(1990).
[2] Norbury C., Nurse P. Curr. Biol. 1:23-24(1991).
[3] Lew D.J., Reed S.I. Trends Cell Biol. 2:77-81(1992).
[4] Nicholas J., Cameron K.R., Honess R.W. Nature 355:362-365(1992).

126. Cystatin domain

This is a very diverse family. Attempts to define separate subfamilies have failed. Typically, either the N-terminal or C-terminal end is very divergent. But splitting into two domains would make very short families. Cathelicidins are related to this family but have not been included. Number of members: 147

Inhibitors of cysteine proteases [1,2,3], which are found in the tissues and body fluids of animals, in the larva of the worm Onchocerca volvulus [4], as well as in plants, can be grouped into three distinct but related families:

- Type 1 cystatins (or stefins), molecules of about 100 amino acid residues with neither disulfide bonds nor carbohydrate groups.
- Type 2 cystatins, molecules of about 115 amino acid residues which contain one or two disulfide loops near their C-terminus.
- Kininogens, which are multifunctional plasma glycoproteins.

They are the precursor of the active peptide bradykinin and play a role in blood coagulation by helping to position optimally prekallikrein and factor XI next to factor XII. They are also inhibitors of cysteine proteases. Structurally, kininogens are made of three contiguous type-2 cystatin domains, followed by an additional domain (of variable length) which contains the sequence of bradykinin. The first of the three cystatin domains seems to have lost its inhibitory activity.

In all these inhibitors, there is a conserved region of five residues which has been proposed to be important for the binding to the cysteine proteases. The consensus pattern starts one residue before this conserved region.

-Consensus pattern: [GSTEQKRV]-Q-[LIVT]-[VAF]-[SAGQ]-G-x-[LIVMNK]-x(2)-[LIVMFY]-x-[LIVMFYA]-[DENQKRHSIV]

[1] Barrett A.J. Trends Biochem. Sci. 12:193-196(1987).
[2] Rawlings N.D., Barrett A.J. J. Mol. Evol. 30:60-71(1990).
[3] Turk V., Bode W. FEBS Lett. 285:213-219(1991).
[4] Lustigman S., Brotman B., Huima T., Prince A.M. Mol. Biochem. Parasitol. 45:65-76(1991).

127. cytochrome_c (Cytochrome c)

The Pfam entry does not include all prosite members.

The cytochrome 556 and cytochrome c' families are not included.

Number of members: 259

In proteins belonging to cytochrome c family [1], the heme group is covalently attached by thioether bonds to two conserved cysteine residues. The consensus sequence for this site is Cys-X-X-Cys-His and the histidine residue is one of the two axial ligands of the heme iron. This arrangement is shared by all proteins known to belong to cytochrome c family, which presently includes cytochromes c, c', c1 to c6, c550 to c556, cc3/Hmc, cytochrome f and reaction center cytochrome c.

-Consensus pattern: C-{CPWHF}-{CPWR}-C-H-{CFYW}

[1] Mathews F.S. Prog. Biophys. Mol. Biol. 45:1-56(1985).

09559326.061302

128. (DAGKa) Diacylglycerol kinase accessory domain (presumed)

Diacylglycerol (DAG) is a second messenger that acts as a protein kinase C activator. This domain is assumed to be an accessory domain: its function is unknown.

[1] Sakane F, Yamada K, Kanoh H, Yokoyama C, Tanabe T, Nature 1990;344:345-348.[2] Sakane F, Imai S, Kai M, Wada I, Kanoh H, J Biol Chem 1996;271:8394-8401.[3] Schaap D, de Widt J, van der Wal J, Vandekerckhove J, van, Damme J, Gussow D, Ploegh HL, van Blitterswijk WJ, van der, Bend RL, FEBS Lett 1990;275:151-158. [4] Kanoh H, Yamada K, Sakane F, Trends Biochem Sci 1990;15:47-50.

129. (DAGKc) Diacylglycerol kinase catalytic domain (presumed)

Diacylglycerol (DAG) is a second messenger that acts as a protein kinase C activator. The catalytic domain is assumed from the finding of bacterial homologues.

[1] Sakane F, Yamada K, Kanoh H, Yokoyama C, Tanabe T, Nature 1990;344:345-348. [2] Sakane F, Imai S, Kai M, Wada I, Kanoh H, J Biol Chem 1996;271:8394-8401. [3] Schaap D, de Widt J, van der Wal J, Vandekerckhove J, van, Damme J, Gussow D, Ploegh

HL, van Blitterswijk WJ, van der, Bend RL, FEBS Lett 1990;275:151-158. [4] Kanoh H, Yamada K, Sakane F, Trends Biochem Sci 1990;15:47-50.

130. D-amino acid oxidases signature(DAO)

D-amino acid oxidase (EC 1.4.3.3) (DAMOX or DAO) is an FAD flavoenzyme that catalyzes the oxidation of neutral and basic D-amino acids into their corresponding keto acids. DAOs have been characterized and sequenced in fungi and vertebrates where they are known to be located in the peroxisomes. D-aspartate oxidase (EC 1.4.3.1) (DASOX) [1] is an enzyme, structurally related to DAO, which catalyzes the same reaction but is active only toward dicarboxylic D-amino acids. In DAO, a conserved histidine has been shown [2] to be important for the enzyme's catalytic activity. The conserved region around this residue has been developed as a signature pattern for these enzymes.

Consensus pattern: [LIVM](2)-H-[NHA]-Y-G-x-[GSA](2)-x-G-x(5)-G-x-A [H is a probable active site residue]o-

[1] Negri A., Ceciliani F., Tedeschi G., Simonic T., Ronchi S. J. Biol. Chem. 267:11865-11871(1992).

[2] Miyano M., Fukui K., Watanabe F., Takahashi S., Tada M., Kanashiro M., Miyake Y. J. Biochem. 109:171-177(1991).

131. DEAD and DEAH box families ATP-dependent helicases signatures

A number of eukaryotic and prokaryotic proteins have been characterized [1,2,3] on the basis of their structural similarity. They all seem to be involved in ATP-dependent, nucleic-acid unwinding. Proteins currently known to belong to this family are: - Initiation factor eIF-4A. Found in eukaryotes, this protein is a subunit of a high molecular weight complex involved in 5'cap recognition and the binding of mRNA to ribosomes. It is an ATP-dependent RNA-helicase. - PRP5 and PRP28. These yeast proteins are involved in various ATP-requiring steps of the pre-mRNA splicing process. - Pl10, a mouse protein expressed specifically during spermatogenesis. - An3, a Xenopus putative RNA helicase, closely related to Pl10. - SPP81/DED1 and DBP1, two yeast proteins probably involved in pre-mRNA splicing and

09853556.051602

related to Pl10. - Caenorhabditis elegans helicase glh-1. - MSS116, a yeast protein required for mitochondrial splicing. - SPB4, a yeast protein involved in the maturation of 25S ribosomal RNA. - p68, a human nuclear antigen. p68 has ATPase and DNA-helicase activities in vitro. It is involved in cell growth and division. - Rm62 (p62), a Drosophila putative RNA helicase related to p68. - DBP2, a yeast protein related to p68. - DHH1, a yeast protein. - DRS1, a yeast protein involved in ribosome assembly. - MAK5, a yeast protein involved in maintenance of dsRNA killer plasmid. - ROK1, a yeast protein. - ste13, a fission yeast protein. - Vasa, a Drosophila protein important for oocyte formation and specification of embryonic posterior structures. - Me31B, a Drosophila maternally expressed protein of unknown function. - dbpA, an Escherichia coli putative RNA helicase. - deaD, an Escherichia coli putative RNA helicase which can suppress a mutation in the rpsB gene for ribosomal protein S2. - rhlB, an Escherichia coli putative RNA helicase. - rhlE, an Escherichia coli putative RNA helicase. - srmB, an Escherichia coli protein that shows RNA-dependent ATPase activity. It probably interacts with 23S ribosomal RNA. - Caenorhabditis elegans hypothetical proteins T26G10.1, ZK512.2 and ZK686.2. - Yeast hypothetical protein YHR065c. - Yeast hypothetical protein YHR169w. - Fission yeast hypothetical protein SpAC31A2.07c. - Bacillus subtilis hypothetical protein yxiN. All these proteins share a number of conserved sequence motifs. Some of them are specific to this family while others are shared by other ATP-binding proteins or by proteins belonging to the helicases `superfamily' [4,E1]. One of these motifs, called the 'D-E-A-D-box', represents a special version of the B motif of ATP-binding proteins. Some other proteins belong to a subfamily which have His instead of the second Asp and are thus said to be 'D-E-A-H-box' proteins [3,5,6,E1]. Proteins currently known to belong to this subfamily are: - PRP2, PRP16, PRP22 and PRP43. These yeast proteins are all involved in various ATP-requiring steps of the pre-mRNA splicing process. - Fission yeast prh1, which my be involved in pre-mRNA splicing. - Male-less (mle), a Drosophila protein required in males, for dosage compensation of X chromosome linked genes. - RAD3 from yeast. RAD3 is a DNA helicase involved in excision repair of DNA damaged by UV light, bulky adducts or cross-linking agents. Fission yeast rad15 (rhp3) and mammalian DNA excision repair protein XPD (ERCC-2) are the homologs of RAD3. - Yeast CHL1 (or CTF1), which is important for chromosome transmission and normal cell cycle progression in G(2)/M. - Yeast TPS1. - Yeast hypothetical protein YKL078w. - Caenorhabditis elegans hypothetical proteins C06E1.10 and K03H1.2. - Poxviruses' early transcription factor 70 Kd subunit which acts with RNA polymerase to initiate transcription from early gene promoters. - I8, a putative vaccinia virus helicase. - hrpA, an Escherichia coli putative RNA helicase. Signature patterns for both subfamilies were developed.

Consensus pattern: [LIVMF](2)-D-E-A-D-[RKEN]-x-[LIVMFYGSTN

Consensus pattern: [GSAH]-x-[LIVMF](3)-D-E-[ALIV]-H-[NECR]

Note: proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop) (see the relevant entry <PDOC00017

[1] Schmid S.R., Linder P. Mol. Microbiol. 6:283-292(1992).
[2] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).
[3] Wassarman D.A., Steitz J.A. Nature 349:463-464(1991).
[4] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).
[5] Harosh I., Deschavanne P. Nucleic Acids Res. 19:6331-6331(1991).
[6] Koonin E.V., Senkevich T.G. J. Gen. Virol. 73:989-993(1992).

132. (DHBP_synthase) 3,4-dihydroxy-2-butanone 4-phosphate synthase

3,4-Dihydroxy-2-butanone 4-phosphate is biosynthesized from ribulose 5-phosphate and serves as the biosynthetic precursor for the xylene ring of riboflavin. Sometimes found as a bifunctional enzyme with GTP_cyclohydro2.

Richter G, Krieger C, Volk R, Kis K, Ritz H, Gotze E, Bacher A, Methods Enzymol 1997;280:374-382.

133. (DHDPS) Dihydrodipicolinate synthetase signatures

Dihydrodipicolinate synthetase (EC 4.2.1.52) (DHDPS) [1] catalyzes, in higher plants chloroplast and in many bacteria (gene dapA), the first reaction specific to the biosynthesis of lysine and of diaminopimelate. DHDPS is responsible for the condensation of aspartate semialdehyde and pyruvate by aping-pong mechanism in which pyruvate first binds to the enzyme by forming a Schiff-base with a lysine residue. Three other proteins are structurally related to DHDPS and probably also act via a similar catalytic mechanism: - Escherichia coli

N-acetylneuraminate lyase (EC 4.1.3.3) (gene nanA), which catalyzes the condensation of N-acetyl-D-mannosamine and pyruvate to form N-acetylneuraminate. - Rhizobium meliloti protein mosA [3], which is involved in the biosynthesis of the rhizopine 3-o-methyl-scyllo-inosamine. - Escherichia coli hypothetical protein yjhH. Two signature patterns for these enzymes were developed . The first one is centered on highly conserved region in the N-terminal part of these proteins. The second signature contains a lysine residue which has been shown, in Escherichia coli dapA [2], to be the one that forms a Schiff-base with the substrate.

Consensus pattern: [GSA]-[LIVM]-[LIVMFY]-x(2)-G-[ST]-[TG]-G-E-[GASNF]-x(6)- [EQ] -

Consensus pattern: Y-[DNS]-[LIVMFA]-P-x(2)-[ST]-x(3)-[LIVMG]-x(13,14)-[LIVM]- x-[SGA]-[LIVMF]-K-[DEQAF]-[STAC] [K is involved in Schiff-base formation]-

[1] Kaneko T., Hashimoto T., Kumpaisal R., Yamada Y. J. Biol. Chem. 265:17451-17455(1990).
[2] Laber B., Gomis-Rueth F.-X., Romao M.J., Huber R. Biochem. J. 288:691-695(1992).
[3] Murphy P.J., Trenz S.P., Grzemski W., de Bruijn F.J., Schell J. J. Bacteriol. 175:5193-5204 (1993).

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134. (DHOdehase) Dihydroorotate dehydrogenase signatures

Dihydroorotate dehydrogenase (EC 1.3.3.1) (DHOdehase) catalyzes the fourth step in the de novo biosynthesis of pyrimidine, the conversion of dihydroorotate into orotate. DHOdehase is a ubiquitous FAD flavoprotein. In bacteria (gene pyrD), DHOdease is located on the inner side of the cytosolic membrane. In some yeasts, such as in Saccharomyces cerevisiae (gene URA1), it is a cytosolic protein while in other eukaryotes it is found in the mitochondria [1]. The sequence of DHOdease is rather well conserved and two signature patterns were developed specific to this enzyme. The first corresponds to a region in the N-terminal section of the enzyme while the second is located in the C-terminal section and seems to be part of the FAD-binding domain.

Consensus pattern[GS]-x(4)-[GK]-[GSTA]-[LIVFSTA]-[GT]-x(3)-[NQR]-x-G-[NHY]-x(2)-P-[RT]

Consensus pattern[LIVM](2)-[GSA]-x-G-G-[IV]-x-[STGDN]-x(3)-[ACV]-x(6)-G-A

[1] Nagy M., Lacroute F., Thomas D. Proc. Natl. Acad. Sci. U.S.A. 89:8966-8970(1992).

135. (DMRL_synthase) 6,7-dimethyl-8-ribityllumazine synthase

136. (DNA_methylase) C-5 cytosine-specific DNA methylases signatures

C-5 cytosine-specific DNA methylases (EC 2.1.1.73) (C5 Mtase) are enzymes that specifically methylate the C-5 carbon of cytosines in DNA [1,2,3]. Such enzymes are found in the proteins described below. - As a component of type II restriction-modification systems in prokaryotes and some bacteriophages. Such enzymes recognize a specific DNA sequence where they methylate a cytosine. In doing so, they protect DNA from cleavage by type II restriction enzymes that recognize the same sequence. The sequences of a large number of type II C-5 Mtases are known. - In vertebrates, there are a number of C-5 Mtases that methylate CpG dinucleotides. The sequence of the mammalian enzyme is known.C-5 Mtases share a number of short conserved regions. Two of them were selected. The first is centered around a conserved Pro-Cys dipeptide in which the cysteine has been shown [4] to be involved in the catalytic mechanism; it appears to form a covalent intermediate with the C6 position of cytosine. The second region is located at the C-terminal extremity in type-II enzymes

Consensus pattern: [DENKS]-x-[FLIV]-x(2)-[GSTC]-x-P-C-x(2)-[FYWLIM]-S [C is the active site residue]-

Consensus pattern: [RKQGTF]-x(2)-G-N-[STAG]-[LIVMF]-x(3)-[LIVMT]-x(3)-[LIVM]-x(3)-[LIVM]-

[1] Posfai J., Bhagwat A.S., Roberts R.J. Gene 74:261-263(1988).
[2] Kumar S., Cheng X., Klimasauskas S., Mi S., Posfai J., Roberts R.J., Wilson G.G. Nucleic Acids Res. 22:1-10(1994).
[3] Lauster R., Trautner T.A., Noyer-Weidner M. J. Mol. Biol. 206:305-312(1989).

[4] Chen L., McMillan A.M., Chang W., Ezak-Nipkay K., Lane W.S., Verdine G.L. Biochemistry 30:11018-11025(1991).

137. (DNAphotolyase) DNA photolyases class 2 signatures

Deoxyribodipyrimidine photolyase (EC 4.1.99.3) (DNA photolyase) [1,2] is a DNArepair enzyme. It binds to UV-damaged DNA containing pyrimidine dimers and, upon absorbing a near-UV photon (300 to 500 nm), breaks the cyclobutane ring joining the two pyrimidines of the dimer. DNA photolyase is an enzyme that requires two choromophore-cofactors for its activity: a reduced FADH2 and either 5,10-methenyltetrahydrofolate (5,10-MTFH) or an oxidized 8-hydroxy-5-deazaflavin (8-HDF) derivative (F420). The folate or deazaflavin chromophore appears to function as an antenna, while the FADH2 chromophore is thought to be responsible for electron transfer. On the basis of sequence similarities[3] DNA photolyases can be grouped into two classes. The second class contains enzymes from Myxococcus xanthus, methanogenic archaebacteria, insects, fish and marsupial mammals. It is not yet known what second cofactor is bound to class 2 enzymes. There are a number of conserved sequence regions in all known class 2 DNAphotolyases, especially in the C-terminal part. Two of these regions were selected as signature patterns.

Consensus pattern: F-x-E-E-x-[LIVM](2)-R-R-E-L-x(2)-N-F-

Consensus pattern: G-x-H-D-x(2)-W-x-E-R-x-[LIVM]-F-G-K-[LIVM]-R-[FY]-M-N-

[1] Sancar G.B., Sancar A. Trends Biochem. Sci. 12:259-261(1987).

[2] Jorns M.S. Biofactors 2:207-211(1990).

[3] Yasui A., Eker A.P.M., Yasuhira S., Yajima H., Kobayashi T., Takao M., Oikawa A. EMBO J. 13:6143-6151(1994).

(DNAphotolyase2) DNA photolyases class 1 signatures

Deoxyribodipyrimidine photolyase (EC 4.1.99.3) (DNA photolyase) [1,2] is a DNA repair enzyme. It binds to UV-damaged DNA containing pyrimidine dimers and ,upon absorbing a near-UV photon (300 to 500 nm), breaks the cyclobutane ring joining the two pyrimidines of the dimer. DNA photolyase is an enzyme that requires two choromophore-cofactors for its activity: a reduced FADH2 and either 5,10-methenyltetrahydrofolate (5,10-MTFH) or an oxidized 8-hydroxy-5-deazaflavin (8-HDF) derivative (F420). The folate or deazaflavin chromophore appears to function as an antenna, while the FADH2 chromophore is thought to be responsible for electron transfer. On the basis of sequence similarities[3] DNA photolyases can be grouped into two classes. The first class contains enzymes from Gram-negative and Gram-positive bacteria, the halophilic archaebacteria Halobacterium halobium, fungi and plants. Class 1 enzymes bind either 5,10-MTHF (E.coli, fungi, etc.) or 8-HDF (S.griseus, H.halobium).This family also includes Arabidopsis cryptochromes 1 (CRY1) and 2 (CRY2),which are blue light photoreceptors that mediate blue light-induced gene expression. There are a number of conserved sequence regions in all known class 1 DNA photolyases, especially in the C-terminal part. Two of these regions were selected as signature patterns

Consensus pattern: T-G-x-P-[LIVM](2)-D-A-x-M-[RA]-x-[LIVM]-
Consensus pattern: [DN]-R-x-R-[LIVM](2)-x-[STA](2)-F-[LIVMFA]-x-K-x-L-x(2,3)- W-[KRQ]-

[1] Sancar G.B., Sancar A. Trends Biochem. Sci. 12:259-261(1987).
[2] Jorns M.S. Biofactors 2:207-211(1990).
[3] Yasui A., Eker A.P.M., Yasuhira S., Yajima H., Kobayashi T., Takao M., Oikawa A. EMBO J. 13:6143-6151(1994).
[4] Lin C., Ahmad M., Cashmore A.R. Plant J. 10:893-902(1996).

138. (DNA_pol_A)
DNA polymerase family A signature

Replicative DNA polymerases (EC 2.7.7.7) are the key enzymes catalyzing the accurate replication of DNA. They require either a small RNA molecule or a protein as a primer for the de novo synthesis of a DNA chain. On the basis of sequence similarities a number of DNA polymerases have been grouped together [1,2,3] under the designation of DNA polymerase family A. The polymerases that belong to this family are listed below.

- Escherichia coli and various other bacterial polymerase I (gene polA).
- Thermus aquaticus Taq polymerase.
- Bacteriophage sp01 polymerase.

- Bacteriophage sp02 polymerase.
- Bacteriophage T5 polymerase.
- Bacteriophage T7 polymerase.
- Mycobacteriophage L5 polymerase.
- Yeast mitochondrial polymerase gamma (gene MIP1).

Five regions of similarity are found in all the above polymerases. One of these conserved regions, known as 'motif B' [1], is located in a domain which, in Escherichia coli polA, has been shown to bind deoxynucleotide triphosphate substrates; it contains a conserved tyrosine which has been shown, by photo- affinity labelling, to be in the active site; a conserved lysine, also part of this motif, can be chemically labelled, using pyridoxal phosphate. This conserved region was used as a signature for this family of DNA polymerases.

Consensus patternR-x(2)-[GSAV]-K-x(3)-[LIVMFY]-[AGQ]-x(2)-Y-x(2)-[GS]-x(3)-[LIVMA] Sequences known to belong to this class detected by the pattern ALL.

[1] Delarue M., Poch O., Todro N., Moras D., Argos P. Protein Eng. 3:461-467(1990).
[2] Ito J., Braithwaite D.K. Nucleic Acids Res. 19:4045-4057(1991).
[3] Braithwaite D.K., Ito J. Nucleic Acids Res. 21:787-802(1993).

139. DNA_pol_viral_C
DNA polymerase (viral) C-terminal domain
Number of members: 128

140. (DNA_topoisoII)
DNA topoisomerase II signature

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break. Topoisomerase II is found in phages, archaebacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits (the product of genes 39, 52 and 60). In prokaryotes and in archaebacteria the enzyme, known as DNA gyrase, consists of two subunits (genes gyrA and gyrB [E2]). In some bacteria, a second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes parC and parE). In eukaryotes, type II topoisomerase is a homodimer.

There are many regions of sequence homology between the different subtypes of topoisomerase II. The relation between the different subunits is shown in the following representation:

```
<----------------About-1400-residues---------------------->

[----------Protein 39-*-----][----Protein 52----]        Phage T4
[----------gyrB-------*-----][--------gyrA----------------]  Prokaryote II
                                              Archaebacteria
[----------parE-------*-----][--------parD----------------]  Prokaryote IV
[--------------------*-----------------------------------]  Eukaryote and
                                              ASF
```

'*': Position of the pattern.

As a signature pattern for this family of proteins, a region that contains a highly conserved pentapeptide was selected. The pattern is located in gyrB, in parE, and in protein 39 of phage T4 topoisomerase.

Consensus pattern[LIVMA]-x-E-G-[DN]-S-A-x-[STAG] Sequences known to belong to this class detected by the pattern ALL.

[1] Sternglanz R. Curr. Opin. Cell Biol. 1:533-535(1990).
[2] Bjornsti M.-A. Curr. Opin. Struct. Biol. 1:99-103(1991).
[3] Sharma A., Mondragon A. Curr. Opin. Struct. Biol. 5:39-47(1995).
[4] Roca J. Trends Biochem. Sci. 20:156-160(1995).

141. (DSPc) Tyrosine specific protein phosphatases signature and profiles

Tyrosine specific protein phosphatases (EC 3.1.3.48) (PTPase) [1 to 5] are enzymes that catalyze the removal of a phosphate group attached to a tyrosine residue. These enzymes are very important in the control of cell growth, proliferation, differentiation and transformation. Multiple forms of PTPase have been characterized and can be classified into two categories: soluble PTPases and transmembrane receptor proteins that contain PTPase domain(s). The currently known PTPases are listed below: Soluble PTPases. - PTPN1 (PTP-1B). - PTPN2 (T-cell PTPase; TC-PTP). - PTPN3 (H1) and PTPN4 (MEG), enzymes that contain an N-terminal band 4.1- like domain (see <PDOC00566>) and could act at junctions between the membrane and cytoskeleton. - PTPN5 (STEP). - PTPN6 (PTP-1C; HCP; SHP) and PTPN11 (PTP-2C; SH-PTP3; Syp), enzymes which contain two copies of the SH2 domain at its N-terminal extremity. The Drosophila protein corkscrew (gene csw) also belongs to this subgroup. - PTPN7 (LC-PTP; Hematopoietic protein-tyrosine phosphatase; HePTP). - PTPN8 (70Z-PEP). - PTPN9 (MEG2). - PTPN12 (PTP-G1; PTP-P19). - Yeast PTP1. - Yeast PTP2 which may be involved in the ubiquitin-mediated protein degradation pathway. - Fission yeast pyp1 and pyp2 which play a role in inhibiting the onset of mitosis. - Fission yeast pyp3 which contributes to the dephosphorylation of cdc2. - Yeast CDC14 which may be involved in chromosome segregation. - Yersinia virulence plasmid PTPAses (gene yopH). - Autographa californica nuclear polyhedrosis virus 19 Kd PTPase.Dual specificity PTPases. - DUSP1 (PTPN10; MAP kinase phosphatase-1; MKP-1); which dephosphorylates MAP kinase on both Thr-183 and Tyr-185. - DUSP2 (PAC-1), a nuclear enzyme that dephosphorylates MAP kinases ERK1 and ERK2 on both Thr and Tyr residues. - DUSP3 (VHR). - DUSP4 (HVH2). - DUSP5 (HVH3). - DUSP6 (Pyst1; MKP-3). - DUSP7 (Pyst2; MKP-X). - Yeast MSG5, a PTPase that dephosphorylates MAP kinase FUS3. - Yeast YVH1. - Vaccinia virus H1 PTPase; a dual specificity phosphatase. Receptor PTPases. Structurally, all known receptor PTPases, are made up of a variable length extracellular domain, followed by a transmembrane region and a C-terminalcatalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectintype III (FN-III) repeats, immunoglobulin-like domains, MAM domains orcarbonic anhydrase-like domains in their extracellular region. The cytoplasmic region generally contains two copies of the PTPAse domain. The first seems to have enzymatic activity, while the second is inactive but seems to affect substrate specificity of the first. In these domains, the catalytic cysteine is generally conserved but some other, presumably important, residues are not. In the following table, the domain structure of known receptor PTPases is shown: Extracellular Intracellular ------------------ ------------- Ig FN-3 CAH MAM PTPaseLeukocyte common antigen (LCA) (CD45) 0 2 0 0 2Leukocyte antigen related (LAR) 3 8 0 0 2 Drosophila DLAR 3 9 0 0 2Drosophila DPTP 2 2 0 0 2PTP-alpha (LRP) 0 0 0 0 2PTP-beta 0 16 0 0 1PTP-gamma 0 1 1 0 2PTP-delta 0 >7 0 0 2 PTP-epsilon 0 0 0 0 2PTP-kappa 1 4 0 1 2PTP-mu 1 4 0 1 2PTP-zeta 0 1 1 0 2PTPase domains consist of about 300 amino acids. There are two conserved cysteines, the second one has been shown to be absolutely required for activity. Furthermore, a number of conserved residues in its immediate vicinity have also been shown to be important. A signature pattern for PTPase domains was derived centered on the active site cysteine. There are three profiles for PTPases, the first one spans the complete domain and is not specific to any subtype. The second profile is specific to dual-specificity PTPases and the third one to the PTP subfamily

Consensus pattern: [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY] [C is the active site residue]-

[1] Fischer E.H., Charbonneau H., Tonks N.K. Science 253:401-406(1991).
[2] Charbonneau H., Tonks N.K. Annu. Rev. Cell Biol. 8:463-493(1992).
[3] Trowbridge I.S. J. Biol. Chem. 266:23517-23520(1991).
[4] Tonks N.K., Charbonneau H. Trends Biochem. Sci. 14:497-500(1989).
[5] Hunter T. Cell 58:1013-1016(1989).

142. (DUF10) Uncharacterized protein family UPF0076 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Goat antigen UK114, a human homolog and the rat corresponding protein which is known as perchloric acid soluble protein (PSP1). PSP1 [2] may inhibit an initiation stage of cell-free protein synthesis. - Mouse heat-responsive protein HRSP12. - Yeast chromosome V hypothetical protein YER057c. - Yeast chromosome IX hypothetical protein YIL051c. - Caenorhabditis elegans hypothetical protein C23G10.2. - Escherichia coli hypothetical protein ycdK. - Escherichia coli hypothetical protein yhaR. - Escherichia coli hypothetical protein yjgF and HI0719, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yoaB. - Bacillus subtilis hypothetical protein yabJ. - Haemophilus influenzae hypothetical protein HI1627. - Helicobacter pylori hypothetical protein HP0944. -

Lactococcus lactis aldR. - Myxococcus xanthus dfrA. - Synechocystis strain PCC 6803 hypothetical protein slr0709. - Rhizobium strain NGR234 symbiotic plasmid hypothetical protein y4sK. - Pyrococcus horikoshii hypothetical protein PH0854.These are small proteins of around 15 Kd whose sequence is highly conserved.As a signature pattern, a well conserved region located in the C-terminal part of these proteins was selected.

Consensus pattern: [PA]-[ASTPV]-R-[SACVF]-x-[LIVMFY]-x(2)-[GSAKR]-x-[LMVA]-x(5,8)-[LIVM]-E-[MI]-

[1] Bairoch A. Unpublished observations (1995).
[2] Oka T., Tsuji H., Noda C., Sakai K., Hong Y.-M., Suzuki I., Munoz S., Natori Y. J. Biol. Chem. 270:30060-30067(1995).

143. (DUF3)Domain of Unknown Function 3

Domain apparently occurring exclusively in eubacteria. Unknown function.

144. (DUF6) Integral membrane protein

This family includes many hypothetical membrane proteins of unknown function. Many of the proteins contain two copies of the aligned region.

145. (DUF7) Integral membrane protein

This family includes many hypothetical membrane proteins of unknown function. Swiss:P14502 has been implicated in resistance to ethidium bromide.

146. (DapB) Dihydrodipicolinate reductase signature

Dihydrodipicolinate reductase (EC 1.3.1.26) catalyzes the second step in the biosynthesis of diaminopimelic acid and lysine, the NAD or NADP-dependent reduction of 2,3-dihydrodipicolinate into 2,3,4,5-tetrahydrodipicolinate. This enzyme is present in bacteria (gene dapB) and higher plants. As a signature pattern the best conserved region in this enzyme was selected. It is located in the central section and is part of the substrate-binding region [1].

Consensus pattern: E-[IV]-x-E-x-H-x(3)-K-x-D-x-P-S-G-T-A-

[1] Scapin G., Blanchard J.S., Sacchettini J.C. Biochemistry 34:3502-3512(1995).

147. DedA family

This family combines the DedA related proteins and YIAN/YGIK family. Members of this family are not functionally characterised. These proteins contain multiple predicted transmembrane regions.

148. DegT/DnrJ/EryC1/StrS family

The members of this family exhibit some characteristics of the sensor protein of two-component signal transduction systems, however none of the members show any sequence similarity to these protein kinases. The members of this family do have the typical helix-turn-helix motif of DNA binding proteins.

[1] Stutzman-Engwall KJ, Otten SL, Hutchinson CR, J Bacteriol 1992;174:144-154.

149. (Desaturase) Fatty acid desaturases signatures

Fatty acid desaturases (EC 1.14.99.-) are enzymes that catalyze the insertion of a double bond at the delta position of fatty acids. There seems to be two distinct families of fatty acid desaturases which do not seem to be evolutionary related. Family 1 is composed of: - Stearoyl-CoA desaturase (SCD) (EC 1.14.99.5) [1]. SCD is a key regulatory enzyme of unsaturated fatty acid biosynthesis. SCD introduces a cis double bond at the delta(9) position of fatty acyl-CoA's such as palmitoleoyl- and oleoyl-CoA. SCD is a membrane-bound enzyme that is thought to function as a part of a multienzyme complex in the endoplasmic reticulum of vertebrates and fungi. As a signature pattern for this family a conserved region in the C-terminal part of these enzymes was selected, this region is rich in histidine residues and in aromatic residues. Family 2 is composed of: - Plants stearoyl-acyl-carrier-protein desaturase (EC 1.14.99.6) [2], these enzymes catalyze the introduction of a double bond at the delta(9) position of steraoyl-ACP to produce oleoyl-ACP. This enzyme is responsible for the conversion of saturated fatty acids to unsaturated fatty acids in the synthesis of vegetable oils. - Cyanobacteria desA [3] an enzyme that can introduce a second cis double bond at the delta(12) position of fatty acid bound to membranes glycerolipids. DesA is involved in chilling tolerance; the phase transition temperature of lipids of cellular membranes being dependent on the degree of unsaturation of fatty acids of the membrane lipids. As a signature pattern for this family a conserved region in the C-terminal part of these enzymes was selected.

Consensus pattern: G-E-x-[FY]-H-N-[FY]-H-H-x-F-P-x-D-Y-
Consensus pattern: [ST]-[SA]-x(3)-[QR]-[LI]-x(5,6)-D-Y-x(2)-[LIVMFYW]-[LIVM]- [DE]-

[1] Kaestner K.H., Ntambi J.M., Kelly T.J. Jr., Lane M.D. J. Biol. Chem. 264:14755-14761(1989).
[2] Shanklin J., Somerville C.R. Proc. Natl. Acad. Sci. U.S.A. 88:2510-2514(1991).
[3] Wada H., Gombos Z., Murata N. Nature 347:200-203(1990).

150. Dihydroorotase signatures

Dihydroorotase (EC 3.5.2.3) (DHOase) catalyzes the third step in the de novo biosynthesis of pyrimidine, the conversion of ureidosuccinic acid (N-carbamoyl-L-aspartate) into dihydroorotate. Dihydroorotase binds a zinc ion which is required for its catalytic activity [1]. In bacteria, DHOase is a dimer of identical chains of about 400 amino-acid residues (gene pyrC). In higher eukaryotes, DHOase is part of a large multi-functional protein known as 'rudimentary' in Drosophila and CAD in mammals and which catalyzes the first three steps of pyrimidine biosynthesis [2]. The DHOase domain is located in the central part of this polyprotein. In yeasts, DHOase is encoded by a monofunctional protein (gene URA4). However, a defective DHOase domain [3] is found in a multifunctional protein (gene URA2)that catalyzes the first two steps of pyrimidine biosynthesis. The comparison of DHOase sequences from various sources shows [4] that there are two highly conserved regions. The first located in the N-terminal extremity contains two histidine residues suggested [3] to be involved in binding the zinc ion. The second is found in the C-terminal part. Signature patterns for both regions have been developed. Allantoinase (EC 3.5.2.5) is the enzyme that hydrolyzes allantoin intoallantoate. In yeast (gene DAL1) [5], it is the first enzyme in the allanto indegradation pathway; in amphibians [6] and fish it catalyzes the second step in the degradation of uric acid. The sequence of allantoinase is evolutionary related to that of DHOases.

Consensus pattern: D-[LIVMFYWSAP]-H-[LIVA]-H-[LIVF]-[RN]-x-[PGANF] [The two H's are probable zinc ligands]-

Consensus pattern: [GA]-[ST]-D-x-A-P-H-x(4)-K-

[1] Brown D.C., Collins K.D. J. Biol. Chem. 266:1597-1604(1991).

[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).

[3] Souciet J.-L., Nagy M., Le Gouar M., Lacroute F., Potier S. Gene 79:59-70(1989).

[4] Guyonvarch A., Nguyen-Juilleret M., Hubert J.-C., Lacroute F. Mol. Gen. Genet. 212:134-141(1988).

[5] Buckholz R.G., Cooper T.G. Yeast 7:913-923(1991).

[6] Hayashi S., Jain S., Chu R., Alvares K., Xu B., Erfurth F., Usuda N., Rao M.S., Reddy S.K., Noguchi T., Reddy J.K., Yeldandi A.Y. J. Biol. Chem. 269:12269-12276(1994).

151. dnaJ domains signatures and profile

The prokaryotic heat shock protein dnaJ interacts with the chaperone hsp70-like dnaK protein [1]. Structurally, the dnaJ protein consists of an N- terminal conserved domain (called 'J' domain) of about 70 amino acids, a glycine-rich region ('G' domain') of about 30 residues, a central domain containing four repeats of a CXXCXGXG motif ('CRR' domain) and a C-terminal region of 120 to 170 residues. Such a structure is shown in the following schematic representation:

+------------+-+-------+-----+-----------+-------------------------------+ | N-terminal | | Gly-R | | CXXCXGXG | C-terminal | +------------+-+-------+-----+-----------+--------------------------------------+

It has been shown [2] that the 'J' domain as well as the 'CRR' domain are also found in other prokaryotic and eukaryotic proteins which are listed below.

a) Proteins containing both a 'J' and a 'CRR' domain:

- Yeast protein MAS5/YDJ1 which seems to be involved in mitochondrial protein import.
- Yeast protein MDJ1, involved in mitochondrial biogenesis and protein folding.
- Yeast protein SCJ1, involved in protein sorting.
- Yeast protein XDJ1.
- Plants dnaJ homologs (from leek and cucumber).
- Human HDJ2, a dnaJ homolog of unknown function.
- Yeast hypothetical protein YNL077w.

b) Proteins containing a 'J' domain without a 'CRR' domain:

- Rhizobium fredii nolC, a protein involved in cultivar-specific nodulation of soybean.
- Escherichia coli cbpA [3], a protein that binds curved DNA.
- Yeast protein SEC63/NPL1, important for protein assembly into the endoplasmic reticulum and the nucleus.
- Yeast protein SIS1, required for nuclear migration during mitosis.
- Yeast protein CAJ1.
- Yeast hypothetical protein YFR041c.
- Yeast hypothetical protein YIR004w.
- Yeast hypothetical protein YJL162c.
- Plasmodium falciparum ring-infected erythrocyte surface antigen (RESA). RESA, whose function is not known, is associated with the membrane skeleton of newly invaded erythrocytes.
- Human HDJ1.
- Human HSJ1, a neuronal protein.
- Drosophila cysteine-string protein (csp).

A signature pattern for the 'J' domain was developed, based on conserved positions in the C-terminal half of this domain. A pattern for the 'CRR' domain, based on the first two copies of that motif was also developed. A profile for the 'J' domain was also developed.

Consensus pattern: [FY]-x(2)-[LIVMA]-x(3)-[FYWHNT]-[DENQSA]-x-L-x-[DN]-x(3)-[KR]-x(2)-[FYI]-

Consensus pattern: C-[DEGSTHKR]-x-C-x-G-x-[GK]-[AGSDM]-x(2)-[GSNKR]-x(4,6)-C-x(2,3)-C-x-G-x-G-

[1] Cyr D.M., Langer T., Douglas M.G. Trends Biochem. Sci. 19:176-181(1994).

[2] Bork P., Sander C., Valencia A., Bukau B. Trends Biochem. Sci. 17:129-129(1992).

[3] Ueguchi C., Kaneda M., Yamada H., Mizuno T. Proc. Natl. Acad. Sci. U.S.A. 91:1054-1058(1994).

152.

153. Dwarfin

This family known as the dwarfins also includes the drosophila protein MAD. The N-terminus of MAD can bind to DNA [2].

[1] Yingling JM, Das P, Savage C, Zhang M, Padgett RW, Wang XF, Proc Natl Acad Sci U S A 1996;93:8940-8944. [2] Kim J, Johnson K, Chen HJ, Carroll S, Laughon A, Nature 1997;388:304-308.

154. Dynein light chain type 1 signature

Dynein is a multisubunit microtubule-dependent motor enzyme that acts as the force generating protein of eukaryotic cilia and flagella. The cytoplasmic isoform of dynein acts as a motor for the intracellular retrograde motility of vesicles and organelles along microtubules. Dynein is composed of a number of ATP-binding large subunits, intermediate size subunits and small subunits. Among the small subunits, there is a family [1,2] of highly conserved proteins which consist of: - Chlamydomonas reinhardtii flagellar outer arm dynein 8 Kd and 11 Kd light chains. - Higher eukaryotes cytoplasmic dynein light chain 1. - Yeast cytoplasmic dynein light chain 1 (gene DYN2 or SLC1). - Caenorhabditis elegans hypothetical dynein light chains M18.2 and T26A5.9.These proteins are have from 89 to 120 amino acids. As a signature pattern, A highly conserved region was selected.

09555856.051500

Consensus pattern: H-x-I-x-G-[KR]-x-F-[GA]-S-x-V-[ST]-[HY]-E -

[1] King S.M., Patel-King R.S. J. Biol. Chem. 270:11445-11452(1995).

[2] Dick T., Ray K., Salz H.K., Chia W. Mol. Cell. Biol. 16:1966-1977(1996).

155. dUTPase

dUTPase hydrolyzes dUTP to dUMP and pyrophosphate.

[1] Cedergren-Zeppezauer ES, Larsson G, Nyman PO, Dauter Z, Wilson KS, Nature 1992;355:740-743. [2] Mol CD, Harris JM, McIntosh EM, Tainer JA, Structure 1996;4:1077-1092.

156. (dCMP cyt deam) Cytidine and deoxycytidylate deaminases zinc-binding region signature

Cytidine deaminase (EC 3.5.4.5) (cytidine aminohydrolase) catalyzes the hydrolysis of cytidine into uridine and ammonia while deoxycytidylatedeaminase (EC 3.5.4.12) (dCMP deaminase) hydrolyzes dCMP into dUMP. Both enzymes are known to bind zinc and to require it for their catalytic activity[1,2]. These two enzymes do not share any sequence similarity with the exception of a region that contains three conserved histidine and cysteine residues which are thought to be involved in the binding of the catalytic zincion. Such a region is also found in other proteins [3,4]: - Yeast cytosine deaminase (EC 3.5.4.1) (gene FCY1) which transforms cytosine into uracil. - Mammalian apolipoprotein B mRNA editing protein, responsible for the postranscriptional editing of a CAA codon into a UAA (stop) codon in the APOB mRNA. - Riboflavin biosynthesis protein ribG, which converts 2,5-diamino-6- (ribosylamino)-4(3H)-pyrimidinone 5'-phosphate into 5-amino-6- (ribosylamino)-2,4(1H,3H)-pyrimidinedione 5'-phosphate. - Bacillus cereus blasticidin-S deaminase (EC 3.5.4.23), which catalyzes the deamination of the cytosine moiety of the antibiotics blasticidin S, cytomycin and acetylblasticidin S. - Bacillus subtilis protein comEB. This protein is required for the binding and uptake of transforming DNA. - Bacillus subtilis hypothetical protein yaaJ. - Escherichia coli hypothetical protein yfhC. - Yeast hypothetical protein YJL035c. A signature pattern for this zinc-binding region was derived.

Consensus pattern: [CH]-[AGV]-E-x(2)-[LIVMFGAT]-[LIVM]-x(17,33)-P-C-x(2,8)-C-x(3)-[LIVM] [The C's and H are zinc ligands

[1] Yang C., Carlow D., Wolfenden R., Short S.A. Biochemistry 31:4168-4174(1992).
[2] Moore J.T., Silversmith R.E., Maley G.F., Maley F. J. Biol. Chem. 268:2288-2291(1993).
[3] Reizer J., Buskirk S., Bairoch A., Reizer A., Saier M.H. Jr. Protein Sci. 3:853-856(1994).
[4] Bhattacharya S., Navaratnam N., Morrison J.R., Scott J., Taylow W.R. Trends Biochem. Sci. 19:105-106(1994).

09559665 .061500

157. Dehydrins signatures

A number of proteins are produced by plants that experience water-stress. Water-stress takes place when the water available to a plant falls below a critical level. The plant hormone abscisic acid (ABA) appears to modulate the response of plant to water-stress. Proteins that are expressed during water-stress are called dehydrins [1,2] or LEA group 2 proteins [3]. The proteins that belong to this family are listed below.

- Arabidopsis thaliana XERO 1, XERO 2 (LTI30), RAB18, ERD10 (LTI45) ERD14 and COR47.
- Barley dehydrins B8, B9, B17, and B18.
- Cotton LEA protein D-11.
- Craterostigma plantagineum dessication-related proteins A and B.
- Maize dehydrin M3 (RAB-17).
- Pea dehydrins DHN1, DHN2, and DHN3.
- Radish LEA protein.
- Rice proteins RAB 16B, 16C, 16D, RAB21, and RAB25.
- Tomato TAS14.
- Wheat dehydrin RAB 15 and cold-shock protein cor410, cs66 and cs120.

Dehydrins share a number of structural features. One of the most notable features is the presence, in their central region, of a continuous run of five to nine serines followed by a cluster of charged residues. Such a region has been found in all known dehydrins so far with the exception of pea dehydrins. A second conserved feature is the presence of two copies of alysine-rich octapeptide; the first copy is located just after the cluster of charged residues that follows the poly-serine region and the second copy is found at the C-terminal extremity. Signature patterns for both regions were derived.

Consensus pattern: S(5)-[DE]-x-[DE]-G-x(1,2)-G-x(0,1)-[KR](4

Consensus pattern: [KR]-[LIM]-K-[DE]-K-[LIM]-P-G-

[1] Close T.J., Kortt A.A., Chandler P.M. Plant Mol. Biol. 13:95-108(1989).
[2] Robertson M., Chandler P.M. Plant Mol. Biol. 19:1031-1044(1992).
[3] Dure L. III, Crouch M., Harada J., Ho T.-H. D., Mundy J., Quatrano R., Thomas T., Sung Z.R. Plant Mol. Biol. 12:475-486(1989).

158. (deoR) Bacterial regulatory proteins, deoR family signature

The many bacterial transcription regulation proteins which bind DNA through a helix-turn-helix' motif can be classified into subfamilies on the basis of sequence similarities. One of these subfamilies groups the following proteins[1,2]: - accR, the Agrobacterium tumefaciens plasmid pTiC58 repressor of opine catabolism and conjugal transfer. - agaR, the Escherichia coli aga operon putative repressor. - deoR, the Escherichia coli deoxyribose operon repressor. - fucR, the Escherichia coli L-fucose operon activator. - gatR, the Escherichia coli galactitol operon repressor. - glpR, the Escherichia coli glycerol-3-phosphate regulon repressor. - gutR (or srlR), the Escherichia coli glucitol operon repressor. - iolR, from Bacillus subtilis. - lacR, the streptococci lactose phosphotransferase system repressor. - spoIIID, the Bacillus subtilis transcription regulator of the sigK gene. - yfjR, an Escherichia coli hypothetical protein. - ygbI, an Escherichia coli hypothetical protein. - yihW, an Escherichia coli hypothetical protein. - yjfQ, an Escherichia coli hypothetical protein. - yjhJ, an Escherichia coli hypothetical protein. The 'helix-turn-helix' DNA-binding motif of these proteins is located in the N-terminal part of the sequence. The pattern used to detect these proteins starts fourteen residues before the HTH motif and ends one residue after it.

Consensus pattern: R-x(3)-[LIVM]-x(3)-[LIVM]-x(16,17)-[STA]-x(2)-T-[LIVMA]- [RH]-[KRNA]-D-[LIVMF]-

[1] von Bodman S., Hayman G.T., Farrand S.K. Proc. Natl. Acad. Sci. U.S.A. 89:643-647(1992).
[2] Bairoch A. Unpublished observations (1993).

159. dsrm

Double-stranded RNA binding motif

[1] Burd CG, Dreyfuss G; Medline: 94310455, Conserved structures and diversity of functions of RNA-binding proteins. Science 1994;265:615-621.

Sequences gathered for seed by HMM_iterative_training Putative motif shared by proteins that bind to dsRNA. At least some DSRM proteins seem to bind to specific RNA targets. Exemplified by Staufen, which is involved in localization of at least five different mRNAs in the early Drosophila embryo. Also by interferon-induced protein kinase in humans, which is part of the cellular response to dsRNA.

Number of members: 116

160. Dynamin family signature

Dynamin [1,2] is a microtubule-associated force-producing protein of 100 Kd which is involved in the production of microtubule bundles and which is able to bind and hydrolyze GTP. Dynamin is structurally related to the following proteins: - Drosophila shibire protein (gene shi) [3]. Shibire is, very probably, the Drosophila cognate of mammalian dynamin. It seems to provide the motor for vesicular transport during endocytosis. - Yeast vacuolar sorting protein VPS1 (or SPO15) [4], a protein which could also be involved in microtubule-associated motility. - Yeast protein MGM1 [5], which is required for mitochondrial genome maintenance. - Yeast protein DNM1, which is involved in endocytosis. - Interferon induced Mx proteins [6,7]. Interferon alpha or beta induce the synthesis of a family of closely related proteins. Most of these proteins are known to confer resistance to influenza viruses and/or rhabdoviruses on transfected mammalian cell in culture. The three motifs found in all GTP-binding proteins are located in the N-terminal part of these proteins. The signature pattern that was developed for these proteins is based on a highly conserved region downstream of the ATP/GTP-binding motif 'A' (P-loop) (see <PDOC00017>).-

Consensus pattern: L-P-[RK]-G-[STN]-[GN]-[LIVM]-V-T-R-

[1] Vallee R.B., Shpetner H.S. Annu. Rev. Biochem. 59:909-932(1990).

[2] Obar R.A., Collins C.A., Hammarback J.A., Shpetner H.S., Vallee R.B. Nature 347:256-261(1990).

[3] van der Bliek A., Meyerowitz E.M. Nature 351:411-414(1991).

[4] Rothman J.H., Raymond C.K., Gilbert T., O'Hara P.J., Stevens T.H. Cell 61:1063-1074(1990).

[5] Jones B.A., Fangman W.L. Genes Dev. 6:380-389(1992).

[6] Arnheiter H., Meier E. New Biol. 2:851-857(1990).

[7] Staeheli P., Pitossi F., Pavlovic J. Trends Cell Biol. 3:268-272(1993).

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161. (dynamin_2) Dynamin central region

This region lies between the GTPase domain, see dynamin, and the pleckstrin homology (PH) domain.

162. E1-E2 ATPases phosphorylation site

E1-E2 ATPases (also known as P-type) are cation transport ATPases which form an aspartyl phosphate intermediate in the course of ATP hydrolysis. ATPases which belong to this family are listed below [1,2,3]. - Fungal and plant plasma membrane (H+) ATPases [reviewed in 4]. - Vertebrate (Na+, K+) ATPases (sodium pump) [reviewed in 5,6]. - Gastric (K+, H+) ATPases (proton pump). - Calcium (Ca++) ATPases (calcium pump) from the sarcoplasmic reticulum (SR), the endoplasmic reticulum (ER) and the plasma membrane. - Copper (Cu++) ATPases (copper pump) which are involved in two human genetic disorders: Menkes syndrome and Wilson disease [7]. - Bacterial potassium (K+) ATPases. - Bacterial cadmium efflux (Cd++) ATPases [reviewed in 8]. - Bacterial magnesium (Mg++) ATPases. - A probable cation ATPase from Leishmania. - fixI, a probable cation ATPase from Rhizobium meliloti, involved in nitrogen fixation. The region around the phosphorylated aspartate residue is perfectly conserved in all these ATPases and can be used as a signature pattern.

Consensus pattern: D-K-T-G-T-[LI]-[TI] [D is phosphorylated]

[1] Green N.M., McLennan D.H. Biochem. Soc. Trans. 17:819-822(1989).
[2] Green N.M. Biochem. Soc. Trans. 17:970-972(1989).
[3] Fagan M.J., Saier M.H. Jr. J. Mol. Evol. 38:57-99(1994).
[4] Serrano R. Biochim. Biophys. Acta 947:1-28(1988).
[5] Fambrough D.M. Trends Neurosci. 11:325-328(1988).
[6] Sweadner K.J. Biochim. Biophys. Acta 988:185-220(1989).
[7] Bull P.C., Cox D.W. Trends Genet. 10:246-251(1994).
[8] Silver S., Nucifora G., Chu L., Misra T.K. Trends Biochem. Sci. 14:76-80(1989).

163. E1_N

E1 Protein, N terminal domain

Number of members: 90

164. (E1_dehydrog) Dehydrogenase E1 component

This family uses thiamine pyrophosphate as a cofactor. This family includes pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase and 2-oxoisovalerate dehydrogenase.

165. (ECH) Enoyl-CoA hydratase/isomerase signature

Enoyl-CoA hydratase (EC 4.2.1.17) (ECH) [1] and 3-2trans-enoyl-CoA isomerase(EC 5.3.3.8) (ECI) [2] are two enzymes involved in fatty acid metabolism. ECH catalyzes the hydratation of 2-trans-enoyl-CoA into 3-hydroxyacyl-CoA and ECI shifts the 3- double bond of the intermediates of unsaturated fatty acid oxidation to the 2-trans position. Most eukaryotic cells have two fatty-acid beta-oxidation systems, one located in mitochondria and the other in peroxisomes. In mitochondria, ECH and ECI are separate yet structurally related monofunctional enzymes. Peroxisomes contain a trifunctional enzyme [3] consisting of an N-terminal domain that bears both ECH and ECI activity, and a C-terminal domain responsible for 3-hydroxyacyl-CoA dehydrogenase (HCDH) activity. In Escherichia coli (gene fadB) and Pseudomonas fragi (gene faoA), ECH and ECI are also part of a multifunctional enzyme which contains both a HCDH and a3-hydroxybutyryl-CoA epimerase domain [4].A number of other proteins have been found to be evolutionary related to the ECH/ECI enzymes or domains: - 3-hydroxbutyryl-coa dehydratase (EC 4.2.1.55) (crotonase), a bacterial enzyme involved in the butyrate/butanol-producing pathway. - Naphthoate synthase (EC 4.1.3.36) (DHNA synthetase) (gene menB) [5], a bacterial enzyme involved in the biosynthesis of menaquinone (vitamin K2). DHNA synthetase converts O-succinyl-benzoyl-CoA (OSB-CoA) to 1,4-dihydroxy- 2-naphthoic acid (DHNA). - 4-chlorobenzoate dehalogenase (EC 3.8.1.6) [6], a Pseudomonas enzyme which catalyzes the conversion of 4-chlorobenzoate-CoA to 4-hydroxybenzoate-CoA. - A Rhodobacter capsulatus protein of unknown function (ORF257) [7]. - Bacillus subtilis putative polyketide biosynthesis proteins pksH and pksI. - Escherichia coli carnitine racemase (gene caiD) [8]. - Escherichia coli hypothetical protein ygfG. - Yeast hypothetical protein YDR036c.As a signature pattern for these enzymes, a conserved region richin glycine and hydrophobic residues was selected.

Consensus pattern: [LIVM]-[STA]-x-[LIVM]-[DENQRHSTA]-G-x(3)-[AG](3)-x(4)-[LIVMST]-x-[CSTA]-[DQHP]-[LIVMFY]-

[1] Minami-Ishii N., Taketani S., Osumi T., Hashimoto T. Eur. J. Biochem. 185:73-78(1989).

[2] Mueller-Newen G., Stoffel W. Biol. Chem. Hoppe-Seyler 372:613-624(1991).

[3] Palosaari P.M., Hiltunen J.K. J. Biol. Chem. 265:2446-2449(1990).

[4] Nakahigashi K., Inokuchi H. Nucleic Acids Res. 18:4937-4937(1990).

[5] Driscoll J.R., Taber H.W. J. Bacteriol. 174:5063-5071(1992).

[6] Babbitt P.C., Kenyon G.L., Matin B.M., Charest H., Sylvestre M., Scholten J.D., Chang K.-H., Liang P.-H., Dunaway-Mariano D. Biochemistry 31:5594-5604(1992).

[7] Beckman D.L., Kranz R.G. Gene 107:171-172(1991).

[8] Eichler K., Bourgis F., Buchet A., Kleber H.-P., Mandrand-Berthelot M.-A. Mol. Microbiol. 13:775-786(1994).

166. (EF1BD) Elongation factor 1 beta/beta'/delta chain signatures

Eukaryotic elongation factor 1 (EF-1) is responsible for the GTP-dependent binding of aminoacyl-tRNAs to the ribosomes [1]. EF-1 is composed of four subunits: the alpha chain which binds GTP and aminoacyl-tRNAs, the gamma chain that probably plays a role in anchoring the complex to other cellular components and the beta and delta (or beta') chains. The beta and delta chains are highly similar proteins that both stimulate the exchange of GDP bound to the alpha chain for GTP [2]. The beta and delta chains are hydrophilic proteins of around 23 to 31 Kd. Their C-terminal part seems important for the nucleotide exchange activity, while the N-terminal section is probably involved in the interaction with the gamma chain. Two signature patterns for this family of proteins were developed. The first corresponds to an acidic region in the central section; the second, to the C-terminal extremity of these proteins

Consensus pattern: [DE]-[DEG]-[DE](2)-[LIVMF]-D-L-F-G-
Consensus pattern: [IV]-Q-S-x-D-[LIVM]-x-A-[FWM]-[NQ]-K-[LIVM]-

[1] Riis B., Rattan I.S., Clark B.F.C., Merrick W.C. Trends Biochem. Sci. 15:420-424(1990).
[2] van Damme H.T.F., Amons R., Karssies R., Timmers C.J., Janssen G.M.C., Moeller W. Biochim. Biophys. Acta 1050:241-247(1990).

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167. (EF1G_domain) Elongation factor 1 gamma, conserved domain

168. (EFG_C) Elongation factor G C-terminus

This family is always found associated with GTP_EFTU. This family includes the carboxyl terminal regions of Elongation factor G, elongation factor 2 and some tetracycline resistance proteins.

169. (EFP) Elongation factor P signature

Elongation factor P (EF-P) [1] is a prokaryotic protein translation factor required for efficient peptide bond synthesis on 70S ribosomes from fMet-tRNAfMet. EF-P is a protein of 21 Kd. It is evolutionary related to yeiP, an hypothetical protein from Escherichia coli. As a signature pattern, a conserved region located in the C-terminal part of these proteins was selected.

Consensus pattern: K-x-[AV]-x(4)-G-x(2)-[LIV]-x-V-P-x(2)-[LIV]-x(2)-G-

[1] Aoki H., Adams S.-L., Turner M.A., Ganoza M.C. Biochimie 79:7-11(1997).

170. (EF TS) Elongation factor Ts signatures

In prokaryotes elongation factor Ts (EF-Ts) is a component of the elongation cycle of protein biosynthesis. It associates with the EF-Tu.GDP complex and induces the exchange of GDP to GTP, it remains bound to the aminoacyl-tRNA.EF-Tu.GTP complex up to the GTP hydrolysis stage on the ribosome [1].EF-Ts is also a component of the chloroplast protein biosynthetic machinery and is encoded in the genome of some algal chloroplast [2]. It is also present in mitochondria [3]. As signature patterns for EF-Ts, two conserved regions located in the N-terminal part of the protein have been selected.

Consensus pattern: L-R-x(2)-T-[GSDNQ]-x-[GS]-[LIVMF]-x(0,1)-[DENKAC]-x-K-[KRNEQS]-A-L-

Consensus pattern: E-[LIVM]-[NV]-[SCV]-[QE]-T-D-F-V-[SA]-[KRN]-

[1] Bubunenko M.G., Kireeva M.L., Gudkov A.T. Biochimie 74:419-425(1992).

[2] Kostrzewa M., Zetsche K. Plant Mol. Biol. 23:67-76(1993).

[3] Xin H., Woriax V.L., Burkhart W.A., Spremulli L.L. J. Biol. Chem. 270:17243-17249(1995).

171. (EMP24_GP25L) emp24/gp25L/p24 family

Members of this family are implicated in bringing cargo forward from the ER and binding to coat proteins by their cytoplasmic domains. Number of members: 30

Paccaud JP, Thomas DY, Bergeron JJ, Nilsson T, J Cell Biol 1998;140:751-765.

172. ENV_polyprotein

ENV polyprotein (coat polyprotein)

Number of members: 224

173. (ERG4_ERG24) Ergosterol biosynthesis ERG4/ERG24 family signatures

Two fungal enzymes involved in ergosterol biosynthesis and which act by reducing double bonds in precursors of ergosterol have been shown to be evolutionary related [1]. These are C-14 sterol reductase (gene ERG24 in budding yeast and erg3 in Neurospora Crassa) and C-24(28) sterol reductase (gene ERG4 in budding yeast and sts1 in fission yeast). Their sequences are also highly related to that of chicken lamin B receptor, which is thought to anchor the lamina to the inner nuclear membrane. These proteins are highly hydrophobic and seem to contain seven or eight transmembrane regions. As signature patterns, two conserved regions were selected. The first one is apparently located in a loop between the fourth and fifth transmembrane regions and the second is in the C-terminal section.

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Consensus pattern: G-x(2)-[LIVM]-[YH]-D-x-[FYW]-x-G-x(2)-L-N-P-R -

Consensus pattern: [LIVM](2)-H-R-x(2)-R-D-x(3)-C-x(2)-K-Y-G-

[1] Lai M.H., Bard M., Pierson C.A., Alexander J.F., Goebl M., Carter G.T., Kirsch D.R. Gene 140:41-49(1994).

174. (ERM) Ezrin/radixin/moesin family

This family of proteins contain a band 4.1 domain (<u>Band_41</u>), at their amino terminus. This family represents the rest of these proteins.

[1] Yonemura S, Hirao M, Doi Y, Takahashi N, Kondo T, Tsukita S, J Cell Biol 1998;140:885-895.

175. ER lumen protein retaining receptor signatures

Proteins that reside in the lumen of the endoplasmic reticulum (ER) contain aC-terminal tetrapeptide (generally K-D-E-L or H-D-E-L) that serves as a signal for their retrieval (retrograde transport) from subsequent compartments of the secretory pathway. The signal is recognized by a receptor molecule that is believed to cycle between the cis side of the Golgi apparatus and the ER [1].This protein is known as the ER lumen protein retaining receptor or also as the 'KDEL receptor'. It has been characterized in a variety of species, including fungi (gene ERD2), plants, Plasmodium, Drosophila and mammals. In mammals two highly related forms of the receptor are known. Structurally, the receptor is a protein of about 220 residues that seems to contain seven transmembrane regions [2]. The N-terminal part (3 residues) is oriented toward the lumen while the C-terminal tail (about 12 residues) is cytoplasmic. There are three lumenal and three cytoplasmic loops. Two signature patterns for these receptors were developed. The first pattern corresponds to the C-terminal half of the first cytoplasmic loop as well as most of the second transmembrane domain. The second pattern is a perfectly conserved decapeptide that corresponds to the central part of the fifth transmembrane domain.

Consensus pattern: G-I-S-x-[KR]-x-Q-x-L-[FY]-x-[LIV](2)-F-x(2)-R-Y-
Consensus pattern: L-E-[SA]-V-A-I-[LM]-P-Q-L-

[1] Pelham H.R.B. Curr. Opin. Cell Biol. 3:585-591(1991).
[2] Townsley F.M., Wilson D.W., Pelham H.R.B. EMBO J. 12:2821-2829(1993).

176. (ETF_beta) Electron transfer flavoprotein beta-subunit signature

The electron transfer flavoprotein (ETF) [1,2] serves as a specific electron acceptor for various mitochondrial dehydrogenases. ETF transfers electrons to the main respiratory chain via ETF-ubiquinone oxidoreductase. ETF is an heterodimer that consist of an alpha and a beta subunit and which bind one molecule of FAD per dimer. A similar system also exists in some bacteria. The beta subunit of ETF is a protein of about 28 Kd which is structurally related to the bacterial nitrogen fixation protein fixA which could play a role in a redox process and feed electrons to ferredoxin. Other related proteins are: - Escherichia coli hypothetical protein ydiQ. - Escherichia coli hypothetical protein ygcR.As a signature pattern for these proteins, a conserved region which is located in the central section was selected.

Consensus pattern: [IVA]-x-[KR]-x(2)-[DE]-[GD]-[GDE]-x(1,2)-[EQ]-x-[LIV]- x(4)-P-x-[LIVM](2)-[TAC]-

[1] Finocchiaro G., Ikeda Y., Ito M., Tanaka K. Prog. Clin. Biol. Res. 321:637-652(1990).

[2] Tsai M.H., Saier M.H. Jr. Res. Microbiol. 146:397-404(1995).

177. Endonuclease III signatures

Escherichia coli endonuclease III (EC 4.2.99.18) (gene nth) [1] is a DNA repair enzyme that acts both as a DNA N-glycosylase, removing oxidized pyrimidines from DNA, and as an apurinic/apyrimidinic (AP) endonuclease, introducing a single-strand nick at the site from which the damaged base was removed. Endonuclease III is an iron-sulfur protein that binds a single 4Fe-4Scluster. The 4Fe-4S cluster does not seem to be important for catalytic activity, but is probably involved in the proper positioning of the enzyme along the DNA strand [2].Endonuclease III is evolutionary related to the following proteins: - Fission yeast endonuclease III homolog (gene nth1) [3]. - Escherichia coli and related protein DNA repair protein mutY, which is an adenine glycosylase. MutY is a larger protein (350 amino acids) than endonuclease III (211 amino acids). - Micrococcus luteus ultraviolet N-glycosylase/AP lyase which initiates repair at cis-syn pyrimidine dimers. - ORF10 in plasmid pFV1 of the thermophilic archaebacteria Methanobacterium thermoformicicum [4]. Restriction methylase m.MthTI, which is encoded by this plasmid, generates 5-methylcytosine which is subject to deamination resulting in G-T mismatches. This protein could correct these mismatches. - Yeast hypothetical protein YAL015c. - Fission yeast hypothetical protein SpAC26A3.02. - Caenorhabditis elegans hypothetical protein R10E4.5. - Methanococcus jannaschii hypothetical protein MJ0613.The 4Fe-4S cluster is bound by four cysteines which are all located in a 17amino acid region at the C-terminal end of endonuclease III. A similar region is also present in the central section of mutY and in the C-terminus of ORF10and of the Micrococcus UV endonuclease. The 4Fe-4S cluster region does not exist in YAL015c. Two signature patterns for these proteins were developed: the first corresponds to the core of the iron-sulfur binding domain, the second corresponds to the best conserved region in the catalytic core of these enzymes.

Consensus pattern: C-x(3)-[KRS]-P-[KRAGL]-C-x(2)-C-x(5)-C [The four C's are 4Fe-4S ligands]-

Consensus pattern: [GST]-x-[LIVMF]-P-x(5)-[LIVMW]-x(2,3)-[LI]-[PAS]-G-V-[GA]- x(3)-[GAC]-x(3)-[LIVM]-x(2)-[SALV]-[LIVMFYW]-[GANK]-

[1] Kuo C.-F., McRee D., Fisher C.L., O'Handley S.F., Cunnigham R.P., Tainer J.A. Science 258:434-440(1992).

[2] Thomson A.J. Curr. Biol. 3:173-174(1993).

[3] Roldan-Arjona T., Anselmino C., Lindahl T. Nucleic Acids. Res. 3307-3312(1996).

[4] Noelling J., van Eeden F.J.M., Eggen R.I.L., de Vos W.M. Nucleic Acids Res. 20:6501-6507(1992).

178. (Epimerase) NAD dependent epimerase/dehydratase family

This family of proteins utilize NAD as a cofactor. The proteins in this family use nucleotide-sugar substrates for a variety of chemical reactions.

[1] Thoden JB, Hegeman AD, Wesenberg G, Chapeau MC, Frey PA, Holden HM, Biochemistry 1997;36:6294-6304.

179. Exonuclease

This family includes a variety of exonuclease proteins, such as ribonuclease T and the epsilon subunit of DNA polymerase III.

[1] Koonin EV, Deutscher MP, Nucleic Acids Res 1993;21:2521-2522.

180. ENTH

ENTH domain

[1] Kay BK, Yamabhai M, Wendland B, Emr SD; Medline: 99156083, Identification of a novel domain shared by putative components of the endocytic and cytoskeletal machinery. Protein Sci 1999;8:435-438.

The ENTH (Epsin N-terminal homology) domain is found in proteins involved in endocytosis and cytoskeletal machinery. The function of the ENTH domain is unknown.

Number of members: 29

181. (eIF-1A) Eukaryotic initiation factor 1A signature

Eukaryotic translation initiation factor 1A (eIF-1A) [1] (formerly known aseiF-4C) is a protein that seems to be required for maximal rate of protein biosynthesis. It enhances ribosome dissociation into subunits and stabilizesthe binding of the initiator Met-tRNA to 40S ribosomal subunits.eIF-1A is a hydrophilic protein of about 15 to 17 Kd. Archaebacteria also seem to possess a eIF-1A homolog. As a signature pattern, a conserved region in the central section of these proteins was selected.

Consensus pattern: [IM]-x-G-x-[GS]-[KRH]-x(4)-[CL]-x-D-G-x(2)-R-x(2)-[RH]-I- x-G

[1] Wei C.-L., Kainuma M., Hershey J.W.B. J. Biol. Chem. 270:22788-22794(1995).

09595392.062502

182. (eIF-5A) Eukaryotic initiation factor 5A hypusine signature

Eukaryotic initiation factor 5A (eIF-5A) (formerly known as eIF-4D) [1,2] is a small protein whose precise role in the initiation of protein synthesis is not known. It appears to promote the formation of the first peptide bond. eIF-5Aseems to be the only eukaryotic protein to contain an hypusine residue. Hypusine is derived from lysine by the post-translational addition of a butylamino group (from spermidine) to the epsilon-amino group of lysine. The hypusine group is essential to the function of eIF-5A.A hypusine-containing protein has been found in archaebacteria such as Sulfolobus acidocaldarius or Methanococcus jannaschii; this protein is highlysimilar to eIF-5A and could play a similar role in protein biosynthesis. The signature developed for eIF-5A is centered around the hypusine residue.

Consensus pattern: [PT]-G-K-H-G-x-A-K [The first K is modified to hypusine]

[1] Park M.H., Wolff E.C., Folk J.E. Biofactors 4:95-104(1993).

[2] Schnier J., Schwelberger H.G., Smit-McBride Z., Kang H.A., Hershey J.W.B. Mol. Cell. Biol. 11:3105-3114(1991).

183. (efhand) S-100/ICaBP type calcium binding protein signature

S-100 are small dimeric acidic calcium and zinc-binding proteins [1] abundant in the brain. They have two different types of calcium-binding sites: a low affinity one with a special structure and a 'normal' EF-hand type high affinity site. The vitamin-D dependent intestinal calcium-binding proteins (ICaBP or calbindin 9 Kd) also belong to this family of proteins, but it does not form dimers. In the past years the sequences of many new members of this family have been determined (for reviews see [2,3,4]); in most cases the function of these proteins is not yet known, although it is becoming clearthat they are involved in cell growth and differentiation, cell cycle regulation and metabolic control. These proteins are: - Calcyclin (Prolactin receptor associated protein (PRA); clatropin; 2a9; 5B10; S100A6). - Calpactin I light chain (p10; p11; 42c; S100A10). - Calgranulin A (cystic fibrosis antigen (CFAg); MIF related protein 8 (MRP- 8); p8; S100A8). - Calgranulin B (MIF related protein 14 (MRP-14); p14; S100A9). - Calgranulin C. - Calgizzarin (S100C). - Placental calcium-binding protein (CAPL) (18a2; peL98; 42a; p9K; MTS1; metastatin; S100A4). - Protein S-100D (S100A5). - Protein S-100E (S100A3). - Protein S-100L (CAN19; S100A2). - Placental protein S-100P (S100E). - Psoriasin (S100A7). - Chemotactic cytokine CP-10 [5]. - Protein MRP-126 [6]. - Trichohyalin [7]. This is a large intermediate filament-associated protein that associates with keratin intermediate filaments (KIF); it contains a S- 100 type domain in its N-terminal extremity. A number of these proteins are known to bind calcium while others are not (p10for example). Our EF-hand detecting pattern will fail to pick those proteins which have lost their calcium-binding properties. A pattern was developed which unambiguously picks up proteins belonging to this family. This pattern spans the region of the EF-hand high affinity site but makes no assumptions on the calcium-binding properties of this site.

Consensus pattern: [LIVMFYW](2)-x(2)-[LK]-D-x(3)-[DN]-x(3)-[DNSG]-[FY]-x- [ES]-[FYVC]-x(2)-[LIVMFS]-[LIVMF]

[1] Baudier J. (In) Calcium and Calcium Binding proteins, Gerday C., Bollis L., Giller R., Eds., pp102-113, Springer Verlag, Berlin, (1988).

[2] Moncrief N.D., Kretsinger R.H., Goodman M. J. Mol. Evol. 30:522-562(1990).

[3] Kligman D., Hilt D.C. Trends Biochem. Sci. 13:437-443(1988).

[4] Schaefer B.W., Wicki R., Engelkamp D., Mattei M.-G., Heizmann C.W. Genomics 25:638-643(1995).

[5] Lackmann M., Cornish C.J., Simpson R.J., Moritz R.L., Geczy C.L. J. Biol. Chem. 267:7499-7504(1992).

[6] Nakano T., Graf T. Oncogene 7:527-534(1992).

[7] Lee S.-C., Kim I.-G., Marekov L.N., O'Keefe E.J., Parry D.A.D., Steinert P.M., J. Biol. Chem. 268:12164-12176(1993).

EF-hand calcium-binding domain

Many calcium-binding proteins belong to the same evolutionary family and share a type of calcium-binding domain known as the EF-hand [1 to 5]. This type of domain consists of a twelve residue loop flanked on both side by a twelve residue alpha-helical domain. In an EF-hand loop the calcium ion is coordinated in a pentagonal bipyramidal configuration. The six residues involved in the binding are in positions 1, 3, 5, 7, 9 and 12; these residues are denoted by X, Y, Z, -Y, -X and -Z. The invariant Glu or Asp at position 12 provides two oxygens for liganding Ca (bidentate ligand).

Listed below are the proteins which are known to contain EF-hand regions. For each type of protein the total number of EF-hand regions known or supposed to exist is indicated between parenthesis. This number does not include regions which clearly have lost their calcium-binding properties, or the atypical low-affinity site (which spans thirteen residues) found in the S-100/ICaBP family of proteins [6].

- Aequorin and Renilla luciferin binding protein (LBP) (Ca=3).
- Alpha actinin (Ca=2). - Calbindin (Ca=4).
- Calcineurin B subunit (protein phosphatase 2B regulatory subunit) (Ca=4).
- Calcium-binding protein from Streptomyces erythraeus (Ca=3?).
- Calcium-binding protein from Schistosoma mansoni (Ca=2?).
- Calcium-binding proteins TCBP-23 and TCBP-25 from Tetrahymena thermophila (Ca=4?). - Calcium-dependent protein kinases (CDPK) from plants (Ca=4).
- Calcium vector protein from amphoxius (Ca=2).
- Calcyphosin (thyroid protein p24) (Ca=4?).
- Calmodulin (Ca=4, except in yeast where Ca=3).
- Calpain small and large chains (Ca=2). - Calretinin (Ca=6).
- Calcyclin (prolactin receptor associated protein) (Ca=2).

- Caltractin (centrin) (Ca=2 or 4).
- Cell Division Control protein 31 (gene CDC31) from yeast (Ca=2?).
- Diacylglycerol kinase (EC 2.7.1.107) (DGK) (Ca=2).
- FAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) from mammals (Ca=1). - Fimbrin (plastin) (Ca=2).
- Flagellar calcium-binding protein (1f8) from Trypanosoma cruzi (Ca=1 or 2).
- Guanylate cyclase activating protein (GCAP) (Ca=3).
- Inositol phospholipid-specific phospholipase C isozymes gamma-1 and delta-1 (Ca=2) [10]. - Intestinal calcium-binding protein (ICaBPs) (Ca=2).
- MIF related proteins 8 (MRP-8 or CFAG) and 14 (MRP-14) (Ca=2).
- Myosin regulatory light chains (Ca=1). - Oncomodulin (Ca=2).
- Osteonectin (basement membrane protein BM-40) (SPARC) and proteins that contains an 'osteonectin' domain (QR1, matrix glycoprotein SC1) (see the entry <PDOC00535>) (Ca=1). - Parvalbumins alpha and beta (Ca=2).
- Placental calcium-binding protein (18a2) (nerve growth factor induced protein 42a) (p9k) (Ca=2).
- Recoverins (visinin, hippocalcin, neurocalcin, S-modulin) (Ca=2 to 3).
- Reticulocalbin (Ca=4). - S-100 protein, alpha and beta chains (Ca=2).
- Sarcoplasmic calcium-binding protein (SCPs) (Ca=2 to 3).
- Sea urchin proteins Spec 1 (Ca=4), Spec 2 (Ca=4?), Lps-1 (Ca=8).
- Serine/threonine protein phosphatase rdgc (EC 3.1.3.16) from Drosophila (Ca=2) - Sorcin V19 from hamster (Ca=2). - Spectrin alpha chain (Ca=2).
- Squidulin (optic lobe calcium-binding protein) from squid (Ca=4).
- Troponins C; from skeletal muscle (Ca=4), from cardiac muscle (Ca=3), from arthropods and molluscs (Ca=2).

There has been a number of attempts [7,8] to develop patterns that pick-up EF-hand regions, but these studies were made a few years ago when not so many different families of calcium-binding proteins were known. Therefore a new pattern was developed which takes into account all published sequences. This pattern includes the complete EF-hand loop as well as the first residue which follows the loop and which seem to always be hydrophobic.

-Consensus pattern: D-x-[DNS]-{ILVFYW}-[DENSTG]-[DNQGHRK]-{GP}-[LIVMC]-[DENQSTAGC]-x(2)-[DE]-[LIVMFYW]

-Note: positions 1 (X), 3 (Y) and 12 (-Z) are the most conserved.

-Note: the 6th residue in an EF-hand loop is, in most cases a Gly, but the number of exceptions to this 'rule' has gradually increased and therefore the pattern should include all the different residues which have been shown to exist in this position in functional Ca-binding sites.

-Note: the pattern will, in some cases, miss one of the EF-hand regions in some proteins with multiple EF-hand domains.

[1] Kawasaki H., Kretsinger R.H. Protein Prof. 2:305-490(1995).[2] Kretsinger R.H. Cold Spring Harbor Symp. Quant. Biol. 52:499-510(1987).
[3] Moncrief N.D., Kretsinger R.H., Goodman M. J. Mol. Evol. 30:522-562(1990).
[4] Nakayama S., Moncrief N.D., Kretsinger R.H. J. Mol. Evol. 34:416-448(1992).
[5] Heizmann C.W., Hunziker W. Trends Biochem. Sci. 16:98-103(1991).
[6] Kligman D., Hilt D.C. Trends Biochem. Sci. 13:437-443(1988).
[7] Strynadka N.C.J., James M.N.G.
Annu. Rev. Biochem. 58:951-98(1989).
[8] Haiech J., Sallantin J. Biochimie 67:555-560(1985).
[9] Chauvaux S., Beguin P., Aubert J.-P., Bhat K.M., Gow L.A., Wood T.M., Bairoch A. Biochem. J. 265:261-265(1990).
[10] Bairoch A., Cox J.A. FEBS Lett. 269:454-456(1990).

184. Enolase signature

Enolase (EC <u>4.2.1.11</u>) is a glycolytic enzyme that catalyzes the dehydration of2-phospho-D-glycerate to phosphoenolpyruvate [1]. It is a dimeric enzyme that requires magnesium both for catalysis and stabilizing the dimer. Enolase is probably found in all organisms that metabolize sugars. In vertebrates, there are three different tissue-specific isozymes: alpha present in most tissues, beta in muscles and gamma found only in nervous tissues. Tau-crystallin, one of the major lens proteins in some fish, reptiles and birds, has been shown [2] to be evolutionary related to enolase. As a signature pattern for enolase, the best conserved region was selected, it is located in the C-terminal third of the sequence.-

Consensus pattern: [LIV](3)-K-x-N-Q-I-G-[ST]-[LIV]-[ST]-[DE]-[STA]

[1] Lebioda L., Stec B., Brewer J.M. J. Biol. Chem. 264:3685-3693(1989).

[2] Wistow G., Piattigorsky J. Science 236:1554-1556(1987).

185. (F-actin_cap_A) F-actin capping protein alpha subunit signatures

The F-actin capping protein binds in a calcium-independent manner to the fast growing ends of actin filaments (barbed end) thereby blocking the exchange of subunits at these ends. Unlike gelsolin and severin this protein does not sever actin filaments. The F-actin capping protein is a heterodimer composed of two unrelated subunits: alpha and beta.The alpha subunit is a protein of about 268 to 286 amino acid residues whose sequence is well conserved in eukaryotic species [1]. As signature patterns two highly conserved regions in the C-terminal section of the alpha subunit were selected.

Consensus pattern: V-H-[FY](2)-E-D-G-N-V

Consensus pattern: F-K-[AE]-L-R-R-x-L-P-

[1] Cooper J.A., Caldwell J.E., Gattermeir D.J., Torres M.A., Amatruda J.F., Casella J.F. Cell Motil. Cytoskeleton 18:204-214(1991).

186. F-box domain

[1] Bai C, Sen P, Hofmann K, Ma L, Goebl M, Harper JW, Elledge SJ, Cell 1996;86:263-274. [2] Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW, Cell 1997;91:209-219.

187. F-protein

Negative factor, (F-Protein) or Nef.

[1] Arold S, Franken P, Strub M-P, Hoh F, Benichou S, Benarous R, Dumas C; Medline: 98035457, The crystal structure of HIV-1 Nef protein bound to the Fyn kinase SH3 domain

suggests a role for this complex in altered T cell receptor signalling Structure 1997;5:1361-1372.

Nef protein accelerates virulent progression of AIDS by its interaction with cellular proteins involved in signal transduction and host cell activation. Nef has been shown to bind specifically to a subset of the Src kinase family.

Number of members: 1013

188. (FAD_binding_2)

Fumarate reductase / succinate dehydrogenase FAD-binding site

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In bacteria two distinct, membrane-bound, enzyme complexes are responsible for the interconversion of fumarate and succinate (EC 1.3.99.1): fumarate reductase (Frd) is used in anaerobic growth, and succinate dehydrogenase (Sdh) is used in aerobic growth. Both complexes consist of two main components: a membrane-extrinsic component composed of a FAD-binding flavoprotein and an iron-sulfur protein; and an hydrophobic component composed of a membrane anchor protein and/or a cytochrome B.

In eukaryotes mitochondrial succinate dehydrogenase (ubiquinone) (EC 1.3.5.1) is an enzyme composed of two subunits: a FAD flavoprotein and and iron-sulfur protein.

The flavoprotein subunit is a protein of about 60 to 70 Kd to which FAD is covalently bound to a histidine residue which is located in the N-terminal section of the protein [1]. The sequence around that histidine is well conserved in Frd and Sdh from various bacterial and eukaryotic species [2] and can be used as a signature pattern.

Consensus patternR-[ST]-H-[ST]-x(2)-A-x-G-G [H is the FAD binding site] Sequences known to belong to this class detected by the pattern ALL.

[1] Blaut M., Whittaker K., Valdovinos A., Ackrell B.A., Gunsalus R.P., Cecchini G. J. Biol. Chem. 264:13599-13604(1989).

[2] Birch-Machin M.A., Farnsworth L., Ackrell B.A., Cochran B., Jackson S., Bindoff L.A., Aitken A., Diamond A.G., Turnbull D.M. J. Biol. Chem. 267:11553-11558(1992).

189. Fatty acid desaturases signatures (FA_desaturase)

Fatty acid desaturases (EC 1.14.99.-) are enzymes that catalyze the insertion of a double bond at the delta position of fatty acids. There seems to be two distinct families of fatty acid desaturases which do not seem to be evolutionary related. Family 1 is composed of: - Stearoyl-CoA desaturase (SCD) (EC 1.14.99.5) [1]. SCD is a key regulatory enzyme of unsaturated fatty acid biosynthesis. SCD introduces a cis double bond at the delta(9) position of fatty acyl-CoA's such as palmitoleoyl- and oleoyl-CoA. SCD is a membrane-bound enzyme that is thought to function as a part of a multienzyme complex in the endoplasmic reticulum of vertebrates and fungi. As a signature pattern for this family a conserved region in the C-terminal part of these enzymes was selected, this region is rich in histidine residues and in aromatic residues. Family 2 is composed of: - Plants stearoyl-acyl-carrier-protein desaturase (EC 1.14.99.6) [2], these enzymes catalyze the introduction of a double bond at the delta(9) position of steraoyl-ACP to produce oleoyl-ACP. This enzyme is responsible for the conversion of saturated fatty acids to unsaturated fatty acids in the synthesis of vegetable oils. - Cyanobacteria desA [3] an enzyme that can introduce a second cis double bond at the delta(12) position of fatty acid bound to membranes glycerolipids. DesA is involved in chilling tolerance; the phase transition temperature of lipids of cellular membranes being dependent on the degree of unsaturation of fatty acids of the membrane lipids. As a signature pattern for this family a conserved region in the C-terminal part of these enzymes was selected.

Consensus pattern: G-E-x-[FY]-H-N-[FY]-H-H-x-F-P-x-D-Y-

Consensus pattern: [ST]-[SA]-x(3)-[QR]-[LI]-x(5,6)-D-Y-x(2)-[LIVMFYW]-[LIVM]- [DE]-

[1] Kaestner K.H., Ntambi J.M., Kelly T.J. Jr., Lane M.D. J. Biol. Chem. 264:14755-14761(1989).

[2] Shanklin J., Somerville C.R. Proc. Natl. Acad. Sci. U.S.A. 88:2510-2514(1991).

[3] Wada H., Gombos Z., Murata N. Nature 347:200-203(1990).

190. Fructose-1-6-bisphosphatase active site (FBPase)

Fructose-1,6-bisphosphatase (EC 3.1.3.11) (FBPase) [1], a regulatory enzyme in gluconeogenesis, catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate. It is involved in many different metabolic pathways and found in most organisms.Sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37) (SBPase) [2] is an enzyme found plant chloroplast and in photosynthetic bacteria that catalyzes the hydrolysis of sedoheptulose 1,7-bisphosphate to sedoheptulose 7-phosphate, a step in the Calvin's reductive pentose phosphate cycle. It is functionally and structurally related to FBPase. In mammalian FBPase, a lysine residue has been shown to be involved in the catalytic mechanism [3]. The region around this residue is highly conserved and can be used as a signature pattern for FBPase and SBPase. It must be noted that, in some bacterial FBPase sequences, the active site lysine is replaced by an arginine

Consensus pattern: [AG]-[RK]-L-x(1,2)-[LIV]-[FY]-E-x(2)-P-[LIVM]-[GSA] [K/R is the active site residue]-

[1] Benkovic S.J., DeMaine M.M. Adv. Enzymol. 53:45-82(1982).

[2] Raines C.A., Lloyd J.C., Willingham N.M., Potts S., Dyer T.A. Eur. J. Biochem. 205:1053-1059(1992).

[3] Ke H., Thorpe C.M., Seaton B.A., Lipscomb W.N., Marcus F. J. Mol. Biol. 212:513-539(1989).

191. FGGY family of carbohydrate kinases signatures *

It has been shown [1] that four different type of carbohydrate kinases seem to be evolutionary related. These enzymes are: - L-fucolokinase (EC 2.7.1.51) (gene fucK). - Gluconokinase (EC 2.7.1.12) (gene gntK). - Glycerokinase (EC 2.7.1.30) (gene glpK). - Xylulokinase (EC 2.7.1.17) (gene xylB). - L-xylulose kinase (EC 2.7.1.53) (gene lyxK).These enzymes are proteins of from 480 to 520 amino acid residues. As consensus patterns for this family of kinases two conserved regionswere selected, one in the central section, the other in the C-terminal section.

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Consensus pattern: [MFYGS]-x-[PST]-x(2)-K-[LIVMFYW]-x-W-[LIVMF]-x-[DENQTKR]-[ENQH]-

Consensus pattern: [GSA]-x-[LIVMFYW]-x-G-[LIVM]-x(7,8)-[HDENQ]-[LIVMF]-x(2)-[AS]-[STAIVM]-[LIVMFY]-[DEQ]-

[1] Reizer A., Deutscher J., Saier M.H. Jr., Reizer J. Mol. Microbiol. 5:1081-1089(1991).

192. FKBP-type peptidyl-prolyl cis-trans isomerase signatures/profile (FKBP)

FKBP [1,2,3] is the major high-affinity binding protein, in vertebrates, for the immunosuppressive drug FK506. It exhibits peptidyl-prolyl cis-trans isomerase activity (EC 5.2.1.8) (PPIase or rotamase). PPIase is an enzyme that accelerates protein folding by catalyzing the cis-trans isomerization of proline imidic peptide bonds in oligopeptides [4].At least three different forms of FKBP are known in mammalian species: - FKBP-12, which is cytosolic and inhibited by both FK506 and rapamycin. - FKBP-13, which is membrane associated and inhibited by both FK506 and rapamycin. - FKBP-25, which is preferentially inhibited by rapamycin. These forms of FKBP are evolutionary related and show extensive similarities[5,6,7] with the following proteins: - Fungal FKBP. - Mammalian hsp binding immunophilin (HBI) (also called p59). HBI is a protein which binds to hsp90 and contains two FKBP-like domains in its N- terminal section - the first of which seems to be functional. - The C-terminal part of the cell-surface protein mip from Legionella; a protein associated with macrophage infection by an unknown mechanism. - Escherichia coli slyD [8], a protein with a N-terminal FKBP domain followed by an histidine-rich metal-binding domain. - Escherichia coli fkpA. - Escherichia coli fklB (FKBP22). - Escherichia coli slpA. - Bacterial trigger factor (Tig). - Streptomyces hygroscopus and chrysomallus FK506-binding protein. - Chlamydia trachomatis 27 Kd membrane protein. - Neisseria meningitidis strain C114 PPiase. - Probable PPiases from Haemophilus influenzae (HI0754), Methanococcus jannaschii (MJ0278 and MJ0825), Pseudomonas fluorescens and Pseudomonase aeruginosa. Two signature patterns for these proteins were developed. One is based on a conserved region in the N-terminus of FKBP, the other is located in the central section. The profile for FKBP spans the complete domain.

Consensus pattern: [LIVMC]-x-[YF]-x-[GVL]-x(1,2)-[LFT]-x(2)-G-x(3)-[DE]- [STAEQK]-[STAN]-

Consensus pattern: [LIVMFY]-x(2)-[GA]-x(3,4)-[LIVMF]-x(2)-[LIVMFHK]-x(2)-G- x(4)-[LIVMF]-x(3)-[PSGAQ]-x(2)-[AG]-[FY]-G--

[1] Tropschug M., Wachter E., Mayer S., Schoenbrunner E.R., Schmid F.X. Nature 346:674-677(1990).

[2] Stein R.L. Curr. Biol. 1:234-236(1991).

[3] Siekierka J.J., Widerrecht G., Greulich H., Boulton D., Hung S.H.Y., Cryan J., Hodges P.J., Sigal N.H. J. Biol. Chem. 265:21011-21015(1990).

[4] Fischer G., Schmid F.X. Biochemistry 29:2205-2212(1990).

[5] Trandinh C.C., Pao G.M., Saier M.H. Jr. FASEB J. 6:3410-3420(1992).

[6] Galat A. Eur. J. Biochem. 216:689-707(1993).

[7] Hacker J., Fischer G. Mol. Microbiol. 10:445456(1993).

[8] Wuelfing C., Lomardero J., Plueckthun A. J. Biol. Chem. 269:2895-2901(1994).

09545350.061502

193. MAPEG family (aka: FLAP/GST2/LTC4S family signature)

The following mammalian proteins are evolutionary related [1]:

- Leukotriene C4 synthase (EC 2.5.1.37) (gene LTC4S), an enzyme that catalyzes the production of LTC4 from LTA4.
- Microsomal glutathione S-transferase II (EC 2.5.1.18) (GST-II) (gene GST2), an enzyme that can also produces LTC4 fron LTA4.
- 5-lipoxygenase activating protein (gene FLAP), a protein that seems to be required for the activation of 5-lipoxygenase.

These are proteins of 150 to 160 residues that contain three transmembrane segments. As a signature pattern, a conserved region between the first and second transmembrane domains was selected.

Consensus patternc: G-x(3)-F-E-R-V-[FY]-x-A-[NQ]-x-N-C

[1] Jakobsson P.-J., Mancini J.A., Ford-Hutchinson A.W. J. Biol. Chem. 271:22203-22210(1996).

194. FMN-dependent alpha-hydroxy acid dehydrogenases active site (FMN_dh)

A number of oxidoreductases that act on alpha-hydroxy acids and which are FMN-containing flavoproteins have been shown [1,2,3] to be structurally related; these enzymes are: - Lactate dehydrogenase (EC 1.1.2.3), which consists of a dehydrogenase domain and a heme-binding domain called cytochrome b2 and which catalyzes the conversion of lactate into pyruvate. - Glycolate oxidase (EC 1.1.3.15) ((S)-2-hydroxy-acid oxidase), a peroxisomal enzyme that catalyzes the conversion of glycolate and oxygen to glyoxylate and hydrogen peroxide. - Long chain alpha-hydroxy acid oxidase from rat (EC 1.1.3.15), a peroxisomal enzyme. - Lactate 2-monooxygenase (EC 1.13.12.4) (lactate oxidase) from Mycobacterium smegmatis, which catalyzes the conversion of lactate and oxygen to acetate, carbon dioxide and water. - (S)-mandelate dehydrogenase from Pseudomonas putida (gene mdlB), which catalyzes the reduction of (S)-mandelate to benzoylformate. The first step in the reaction mechanism of these enzymes is the abstraction of the proton from the alpha-carbon of the substrate producing a carbanion which can subsequently attach to the N5 atom of FMN. A conserved histidine has been shown [4] to be involved in the removal of the proton. The region around this active site residue is highly conserved and contains an arginine residue which is involved in substrate binding.

Consensus pattern: S-N-H-G-[AG]-R-Q [H is the active site residue] [R is a substrate-binding residue]-

[1] Giegel D.A., Williams C.H. Jr., Massey V. J. Biol. Chem. 265:6626-6632(1990).

[2] Tsou A.Y., Ransom S.C., Gerlt J.A., Buechter D.D., Babbitt P.C., Kenyon G.L. Biochemistry 29:9856-9862(1990).

[3] Le K.H.D., Lederer F. J. Biol. Chem. 266:20877-20880(1991).

[4] Lindqvist Y., Branden C.-I. J. Biol. Chem. 264:3624-3628(1989).

195. Flavin-binding monooxygenase-like (FMO-like)

This family includes FMO proteins, cyclohexanone monooxygenase

196. (FPGS)

Folylpolyglutamate synthase signatures (aka Mur_ligase)

Folylpolyglutamate synthase (EC 6.3.2.17) (FPGS) [1] is the enzyme of folate metabolism that catalyzes ATP-dependent addition of glutamate moieties to tetrahydrofolate.

Its sequence is moderately conserved between prokaryotes (gene folC) and eukaryotes. We developed two signature patterns based on the conserved regions which are rich in glycine residues and could play a role in the catalytical activity and/or in substrate binding.

Consensus pattern [LIVMFY]-x-[LIVM]-[STAG]-G-T-[NK]-G-K-x-[ST]-x(7)- [LIVM](2)-x(3)-[GSK] Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern[LIVMFY](2)-E-x-G-[LIVM]-[GA]-G-x(2)-D-x-[GST]-x-[LIVM](2) Sequences known to belong to this class detected by the pattern ALL.

[1] Shane B., Garrow T., Brenner A., Chen L., Choi Y.J., Hsu J.C., Stover P. Adv. Exp. Med. Biol. 338:629-634(1993).

□

09555356.030702

197. FYVE zinc finger

The FYVE zinc finger is named after four proteins that it has been found in: Fab1, YOTB/ZK632.12, Vac1, and EEA1. The FYVE finger has been shown to bind two Zn++ ions [1]. The FYVE finger has eight potential zinc coordinating cysteine positions. Many members of this family also include two histidines in a motif R+HHC+XCG, where + represents a charged residue and X any residue. Members were included which do not conserve these histidine residues but are clearly related.

[1] Stenmark H, Aasland R, Toh BH, D'Arrigo A, J Biol Chem 1996;271:24048-24054. [2] Gaullier JM, Simonsen A, D'Arrigo A, Bremnes B, Stenmark H, Aasland R, Nature 1998;394:432-433.

198. F_actin_cap_B

F-actin capping protein beta subunit signature

The F-actin capping protein binds in a calcium-independent manner to the fast growing ends of actin filaments (barbed end) thereby blocking the exchange of subunits at these ends. Unlike gelsolin and severin this protein does not sever actin filaments. The F-actin capping protein is a heterodimer composed of two unrelated subunits: alpha and beta.

The beta subunit is a protein of about 280 amino acid residues whose sequence is well conserved in eukaryotic species [1]. As a signature pattern a conserved hexapeptide in the N-terminal section of the beta subunit was selected.

Consensus pattern: C-D-Y-N-R-D Sequences known to belong to this class detected by the pattern ALL.

[1] Amatruda J.F., Cannon J.F., Tatchell K., Hug C., Cooper J.A. Nature 344:352-354(1990).

199. Isopenicillin N synthetase signatures (Fe_Asc_oxidored)

Isopenicillin N synthetase (IPNS) [1,2] is a key enzyme in the biosynthesis of penicillin and cephalosporin. In the presence of oxygen, it removes iron and ascorbate, four hydrogen atoms from L-(alpha-aminoadipyl)-L-cysteinyl-d-valine to form the azetidinone and thiazolidine rings of isopenicillin. IPNS is an enzyme of about 330 amino-acid residues. Two cysteines are conserved in fungal and bacterial IPNS sequences; these may be involved in iron-binding and/or substrate-binding. Cephalosporium acremonium DAOCS/DACS [3] is a bifunctional enzyme involved in cephalosporin biosynthesis. The DAOCS domain, which is structurally related to IPNS, catalyzes the step from penicillin N to deacetoxy-cephalosporin C - used as a substrate by DACS to form deacetylcephalosporin C. Streptomycesclavuligerus possesses a monofunctional DAOCS enzyme (gene cefE) [4] also related to IPNS. Two signature patterns for these enzymes were derived, centered around the conserved cysteine residues.

Consensus pattern: [RK]-x-[STA]-x(2)-S-x-C-Y-[SL]-

Consensus pattern: [LIVM](2)-x-C-G-[STA]-x(2)-[STAG]-x(2)-T-x-[DNG]-

[1] Martin J.F. Trends Biotechnol. 5:306-308(1987).
[2] Chen G., Shiffman D., Mevarech M., Aharonowitz Y. Trends Biotechnol. 8:105-111(1990).
[3] Samson S.M., Dotzlaf J.E., Slisz M.L., Becker G.W., van Frank R.M., Veal L.E., Yeh W.K., Miller J.R., Queener S.W., Ingolia T.D. Bio/Technology 5:1207-1214(1987).
[4] Kovacevic S., Weigel B.J., Tobin M.B., Ingolia T.D., Miller J.R. J. Bacteriol. 171:754-760(1989).

200. Fibrillarin signature

Fibrillarin [1] is a component of a nucleolar small nuclear ribonucleoprotein(SnRNP) particle thought to participate in the first step of the processing of pre-rRNA. In mammals, fibrillarin is associated with the U3, U8 and U13small nuclear RNAs [2]. Fibrillarin is an extremely well conserved protein of about 320 amino acid residues. Structurally it consists of three different domains: - An N-terminal domain of about 80 amino acids which is very rich in glycine and contains a number of dimethylated arginine residues (DMA). - A central domain of about 90 residues which resembles that of RNA-binding proteins and contains an octameric sequence similar to the RNP-2 consensus found in such proteins. - A C-terminal alpha-helical domain. A protein evolutionary related to fibrillarin has been found [3] in archaebacteria such as Methanococcus vannielii or voltae. This protein (geneflpA) is involved in pre-rRNA processing. It lacks the Gly/Arg-rich N-terminal domain. As a signature pattern, a region was selected that starts with and encompases theRNP-2 like octapeptide sequence.

Consensus pattern: [GST]-[LIVMAP]-V-Y-A-[IV]-E-[FY]-[SA]-x-R-x(2)-R-[DE] -

[1] Aris J.P., Blobel G. Proc. Natl. Acad. Sci. U.S.A. 88:931-935(1991).
[2] Bandziulis R.J., Swanson M.S., Dreyfuss G. Genes Dev. 3:431-437(1989).
[3] Agha-Amiri K. J. Bacteriol. 176:2124-2127(1994).

201. Filamin/ABP280 repeat

[1] Fucini P, Renner C, Herberhold C, Noegel AA, Holak TA, Nat Struct Biol 1997;4:223-230.

202. Fucosyl transferase

This family of Fucosyltransferases are the enzymes transferring fucose from GDP-Fucose to GlcNAc in an alpha1,3 linkage [1].

[1] Breton C, Oriol R, Imberty A; Glycobiology 1998;8:87-94.

203. 2Fe-2S ferredoxins, iron-sulfur binding region signature (fer2A)

Ferredoxins [1] are a group of iron-sulfur proteins which mediate electron transfer in a wide variety of metabolic reactions. Ferredoxins can be divided into several subgroups depending upon the physiological nature of the iron sulfur cluster(s) and according to sequence similarities. One of these subgroups are the 2Fe-2S ferredoxins, which are proteins or domains of around one hundred amino acid residues that bind a single 2Fe-2S iron-sulfur cluster. The proteins that are known [2] to belong to this family are listed below. - Ferredoxin from photosynthetic organisms; namely plants and algae where it is located in the chloroplast or cyanelle; and cyanobacteria. - Ferredoxin from archaebacteria of the Halobacterium genus. - Ferredoxin IV (gene pftA) and V (gene fdxD) from Rhodobacter capsulatus. - Ferredoxin in the toluene degradation operon (gene xylT) and naphthalene degradation operon (gene nahT) of Pseudomonas putida. - Hypothetical Escherichia coli protein yfaE. - The N-terminal domain of the bifunctional ferredoxin/ferredoxin reductase electron transfer component of the benzoate 1,2-dioxygenase complex (gene benC) from Acinetobacter calcoaceticus, the toluene 4-monooxygenase complex (gene tmoF), the toluate 1,2-dioxygenase system (gene xylZ), and the xylene monooxygenase system (gene xylA) from Pseudomonas. - The N-terminal domain of phenol hydroxylase protein p5 (gene dmpP) from Pseudomonas Putida. - The N-terminal domain of methane monooxygenase component C (gene mmoC) from Methylococcus capsulatus . - The C-terminal domain of the vanillate degradation pathway protein vanB in a Pseudomonas species. - The N-terminal domain of bacterial fumarate reductase iron-sulfur protein (gene frdB). - The N-terminal domain of CDP-6-deoxy-3,4-glucoseen reductase (gene ascD) from Yersinia pseudotuberculosis. - The central domain of eukaryotic succinate dehydrogenase (ubiquinone) iron- sulfur protein. - The N-terminal

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domain of eukaryotic xanthine dehydrogenase. - The N-terminal domain of eukaryotic aldehyde oxidase. In the 2Fe-2S ferredoxins, four cysteine residues bind the iron-sulfur cluster. Three of these cysteines are clustered together in the same region of the protein. Our signature pattern spans that iron-sulfur binding region.

Consensus pattern: C-{C}-{C}-[GA]-{C}-C-[GAST]-{CPDEKRHFYW}-C [The three C's are 2Fe-2S ligands]-

[1] Meyer J. Trends Ecol. Evol. 3:222-226(1988).[2] Harayama S., Polissi A., Rekik M. FEBS Lett. 285:85-88(1991).

Adrenodoxin family, iron-sulfur binding region signature (fer2B)

Ferredoxins [1] are a group of iron-sulfur proteins which mediate electron transfer in a wide variety of metabolic reactions. Ferredoxins can be divided into several subgroups depending upon the physiological nature of the iron sulfur cluster(s) and according to sequence similarities. One family of ferredoxins groups together the following proteins that all bind a single 2Fe-2S iron-sulfur cluster: - Adrenodoxin (ADX) (adrenal ferredoxin), a vertebrate mitochondrial protein which transfers electrons from adrenodoxin reductase to cytochrome P450scc, which is involved in cholesterol side chain cleavage. - Putidaredoxin (PTX), a Pseudomonas putida protein which transfers electrons from putidaredoxin reductase to cytochrome P450-cam, which is involved in the oxidation of camphor. - Terpredoxin [2], a Pseudomonas protein which transfers electrons from terpredoxin reductase to cytochrome P450-terp, which is involved in the oxidation of alpha-terpineol. - Rhodocoxin [3], a Rhodococcus protein which transfers electrons from rhodocoxin reductase to cytochrome CYP116 (thcB), which is involved in the degradation of thiocarbamate herbicides. - Escherichia coli ferredoxin (gene fdx) [4] whose exact function is not yet known. - Rhodobacter capsulatus ferredoxin VI [5], which may transfer electrons to a yet uncharacterized oxygenase. - Caulobacter crescentus ferredoxin (gene fdxB) [6].In these proteins, four cysteine residues bind the iron-sulfur cluster. Three of these cysteines are clustered together in the same region of the protein. Our signature pattern spans that iron-sulfur binding region.

09565365.072600

Consensus pattern: C-x(2)-[STAQ]-x-[STAMV]-C-[STA]-T-C-[HR] [The three C's are 2Fe-2S ligands]-

[1] Meyer J. Trends Ecol. Evol. 3:222-226(1988).
[2] Peterson J.A., Lu J.-Y., Geisselsoder J., Graham-Lorence S., Carmona C., Witney F., Lorence M.C. J. Biol. Chem. 267:14193-14203(1992).
[3] Nagy I., Schoofs G., Compernolle F., Proost P., Vanderleyden J., De Mot R. J. Bacteriol. 177:676-687(1995).
[4] Ta D.T., Vickery L.E. J. Biol. Chem. 267:11120-11125(1992).
[5] Naud I., Vincon M., Garin J., Gaillard J., Forest E., Jouanneau Y. Eur. J. Biochem. 222:933-939(1994).
[6] Amemiya K EMBL/Genbank: X51607.

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204. 4Fe-4S ferredoxins, iron-sulfur binding region signature (fer4)

Ferredoxins [1] are a group of iron-sulfur proteins which mediate electron transfer in a wide variety of metabolic reactions. Ferredoxins can be divided into several subgroups depending upon the physiological nature of the iron-sulfur cluster(s). One of these subgroups are the 4Fe-4S ferredoxins, which are found in bacteria and which are thus often referred as 'bacterial-type' ferredoxins. The structure of these proteins [2] consists of the duplication of a domain of twenty six amino acid residues; each of these domains contains four cysteine residues that bind to a 4Fe-4S center. A number of proteins have been found [3] that include one or more 4Fe-4Sbinding domains similar to those of bacterial-type ferredoxins. These proteins are listed below (references are only provided for recently determined sequences). - The iron-sulfur proteins of the succinate dehydrogenase and the fumarate reductase complexes (EC 1.3.99.1). These enzyme complexes, which are components of the tricarboxylic acid cycle, each contain three subunits: a flavoprotein, an iron-sulfur protein, and a b-type cytochrome. The iron- sulfur proteins contain three different iron-sulfur centers: a 2Fe-2S, a 3Fe-3S and a 4Fe-4S. - Escherichia coli anaerobic glycerol-3-phosphate dehydrogenase (EC•1.1.99.5) This enzyme is composed of three subunits: A, B, and C. The C subunit seems to be an iron-sulfur protein with two ferredoxin-like domains in the N-terminal part of the protein. - Escherichia coli anaerobic dimethyl sulfoxide reductase. The B subunit of this enzyme (gene dmsB) is an iron-sulfur protein with four 4Fe-4S ferredoxin-like domains. - Escherichia coli formate hydrogenlyase. Two of the subunits of this oligomeric complex (genes hycB and hycF) seem to be iron-sulfur proteins that each contain two 4Fe-4S ferredoxin-like domains. - Methanobacterium formicicum formate dehydrogenase (EC 1.2.1.2). This enzyme is used by the archaebacteria to grow on formate. The beta chain of this dimeric enzyme probably binds two 4Fe-4S centers. - Escherichia coli formate dehydrogenases N and O (EC 1.2.1.2). The beta chain of these two enzymes (genes fdnH and fdoH) are iron-sulfur proteins with four 4Fe-4S ferredoxin-like domains. - Desulfovibrio periplasmic [Fe] hydrogenase (EC 1.18.99.1). The large chain of this dimeric enzyme binds three 4Fe-4S centers, two of which are located in the ferredoxin-like N-terminal region of the protein. - Methanobacterium thermoautrophicum methyl viologen-reducing hydrogenase subunit mvhB, which contains six tandemly repeated ferredoxin-like domains and which probably binds twelve 4Fe-4S centers. - Salmonella typhimurium anaerobic sulfite reductase (EC 1.8.1.-) [4]. Two of the subunits of this enzyme (genes asrA and asrC) seem to both bind two 4Fe-4S centers. - A Ferredoxin-like protein (gene fixX) from the nitrogen-fixation genes locus of various Rhizobium species, and one from the Nif-region of Azotobacter species. - The 9 Kd polypeptide of chloroplast photosystem I [5] (gene psaC). This protein contains two low potential 4Fe-4S centers, referred as the A and B centers. - The chloroplast frxB protein which is predicted to carry two 4Fe-4S centers. - An ferredoxin from a primitive eukaryote, the enteric amoeba Entamobea histolytica. - Escherichia coli hypothetical protein yjjW, a protein with a N-terminal region belonging to the radical activating enzymes family (see <PDOC00834>) and two potential 4Fe-4S centers.The pattern of cysteine residues in the iron-sulfur region is sufficient todetect this class of 4Fe-4S binding proteins.

Consensus pattern: C-x(2)-C-x(2)-C-x(3)-C-[PEG] [The four C's are 4Fe-4S ligands]-

[1] Meyer J. Trends Ecol. Evol. 3:222-226(1988).
[2] Otaka E., Ooi T. J. Mol. Evol. 26:257-267(1987).
[3] Beinert H. FASEB J. 4:2483-2492(1990).
[4] Huang C.J., Barrett E.L. J. Bacteriol. 173:1544-1553(1991).
[5] Knaff D.B. Trends Biochem. Sci. 13:460-461(1988).

205. NifH/frxC family signatures (fer4_NifH)

Nitrogenase (EC 1.18.6.1) [1] is the enzyme system responsible for biological nitrogen fixation. Nitrogenase is an oligomeric complex which consists of two components: component 1 which contains the active site for the reduction of nitrogen to ammonia and component 2 (also called the iron protein).Component 2 is a homodimer of a protein (gene nifH) which binds a single 4Fe-4S iron sulfur cluster [2]. In the nitrogen fixation process nifH is first reduced by a protein such as ferredoxin; the reduced protein then transfers electrons to component 1 with the concomitant consumption of ATP.A number of proteins are known to be evolutionary related to nifH. These proteins are: - Chloroplast encoded frxC (or chlL) protein [3]. FrxC is encoded on the chloroplast genome of some plant species, its exact function is not known, but it could act as an electron carrier in the conversion of protochlorophyllide to chlorophyllide. - Rhodobacter capsulatus proteins bchL and bchX [4]. These proteins are also likely to play a role in chlorophyll synthesis. There are a number of conserved regions in the sequence of these proteins: in the N-terminal section there is an ATP-binding site motif 'A' (P-loop) and in the central section there are two conserved cysteines which have been shown, in nifH, to be the ligands of the 4Fe-4S cluster. Two signatures patterns that correspond to the regions around these cysteines were developed.

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Consensus pattern: E-x-G-G-P-x(2)-[GA]-x-G-C-[AG]-G [C binds the iron-sulfur center]-
Consensus pattern: D-x-L-G-D-V-V-C-G-G-F-[AG]-x-P [C binds the iron-sulfur center]-

[1] Pau R.N. Trends Biochem. Sci. 14:183-186(1989).
[2] Georgiadis M.M., Komiya H., Chakrabarti P., Woo D., Kornuc J.J., Rees D.C. Science 257:1653-1659(1992).
[3] Fujita Y., Takahashi Y., Kohchi T., Ozeki H., Ohyama K., Matsubara H. Plant Mol. Biol. 13:551-561(1989).
[4] Burke D.H., Alberti M., Hearst J.E. J. Bacteriol. 175:2407-2413(1993).

206. Ferritin iron-binding regions signatures

Ferritin [1,2] is one of the major non-heme iron storage proteins. It consists of a mineral core of hydrated ferric oxide, and a multi-subunit protein shell which englobes the former and assures its solubility in an aqueous environment. In animals the protein is mainly cytoplasmic and there are generally two or more genes that encodes for closely related subunits (in mammals there are two subunits which are known as H(eavy) and L(ight)). In plants ferritin is found in the chloroplast [3].There are a number of well conserved region in the sequence of ferritins. Two of these regions to develop signature patterns were selected. The first pattern is located in the central part of the sequence of ferritin and it contains three conserved glutamate which are thought to be involved in the binding of iron. The second pattern is located in the C-terminal section, it corresponds to a region which forms a hydrophilic channel through which small molecules and ions can gain access to the central cavity of the molecule; this pattern also includes conserved acidic residues which are potential metal-binding sites.

Consensus pattern: E-x-[KR]-E-x(2)-E-[KR]-[LF]-[LIVMA]-x(2)-Q-N-x-R-x-G-R [The 3 E's are potential iron ligands]-

Consensus pattern: D-x(2)-[LIVMF]-[STAC]-[DH]-F-[LI]-[EN]-x(2)-[FY]-L-x(6)- [LIVM]-[KN] [The second D and the E are potential iron ligands]-

[1] Crichton R.R., Charloteaux-Wauters M. Eur. J. Biochem. 164:485-506(1987).
[2] Theil E.C. Annu. Rev. Biochem. 56:289-315(1987).
[3] Ragland M., Briat J.-F., Gagnon J., Laulhere J.-P., Massenet O., Theil E.C. J. Biol. Chem. 265:18339-18344(1990).

207. Intermediate filaments signature (filament)

Intermediate filaments (IF) [1,2,3] are proteins which are primordial components of the cytoskeleton and the nuclear envelope. They generally form filamentous structures 8 to 14 nm wide. IF proteins are members of a very large multigene family of proteins which has been subdivided in five major subgroups: - Type I: Acidic cytokeratins. - Type II: Basic cytokeratins. - Type III: Vimentin, desmin, glial fibrillary acidic protein (GFAP), peripherin, and plasticin. - Type IV: Neurofilaments L, H and M, alpha-internexin and nestin. - Type V: Nuclear lamins A, B1, B2 and C. All IF proteins are structurally similar in that they consist of: a central rod domain comprising some 300 to 350 residues which is arranged in coiled-coiled alpha-helices, with at least two short characteristic interruptions; a N-terminal non-helical domain (head) of variable length; and a C-terminal domain (tail) which is also non-helical, and which shows extreme length variation between different IF proteins. While IF proteins are evolutionary and structurally related, they have limited sequence homologies except in several regions of the rod domain. A conserved region at the C-terminal extremity of the rod domain was used as a sequence pattern for this class of proteins.

Consensus pattern: [IV]-x-[TACI]-Y-[RKH]-x-[LM]-L-[DE]-

[1] Quinlan R., Hutchison C., Lane B. Protein Prof. 2:801-952(1995).
[2] Steiner P.M., Roop D.R. Annu. Rev. Biochem. 57:593-625(1988).
[3] Stewart M. Curr. Opin. Cell Biol. 2:91-100(1990).

208. Flavodoxin signature

Flavodoxins [1,E1] are electron-transfer proteins that function in various electron transport systems. Flavodoxins bind one FMN molecule, which serves as a redox-active prosthetic group. Flavodoxins are functionally interchangeable with ferredoxins. They have been isolated from prokaryotes, cyanobacteria, and some eukaryotic algae. The signature pattern for these proteins is derived from a conserved region in their N-terminal section, this region is involved in the binding of the FMN phosphate group.

Consensus pattern: [LIV]-[LIVFY]-[FY]-x-[ST]-x(2)-[AGC]-x-T-x(3)-A-x(2)-[LIV]-

[1] Wakabayashi S., Kimura K., Matsubara H., Rogers L.J. Biochem. J. 263:981-984(1989).

209. Growth factor and cytokines receptors family signatures (fn3)

A number of receptors for lymphokines, hematopoeitic growth factors and growth hormone-related molecules have been found [1 to 5] to share a common binding domain. Receptors known to belong to this family are: - Cytokine receptor common beta chain. This chain is common to the IL-3, IL-5 and GM-CSF receptors. - Cytokine receptor common gamma chain. This chain is common to the IL-2, IL-4, IL-7 and IL-13 receptors. - Ciliary neurotrophic factor receptor (CNTFR). - Erythropoietin receptor (EPOR). - Granulocyte colony-stimulating factor receptor (G-CSFR). - Granulocyte-macrophage colony-stimulating factor receptor alpha chain (GM- CSFR). - Interleukin-2 receptor beta chain (IL2R-beta). - Interleukin-3 receptor alpha chain (IL3R). - Interleukin-4 receptor alpha chain (IL4R). -

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Interleukin-5 receptor alpha chain (IL5R). - Interleukin-6 receptor (IL6R). - Interleukin-7 receptor alpha chain (IL7R). - Interleukin-9 receptor (IL9R). - Growth hormone receptor (GRHR). - Prolactin receptor (PRLR). - Thrombopoeitin receptor (TPOR).The conserved region constitutes all or part of the extracellular ligand-binding region and is about 200 amino acid residues long. In the N-terminal of this domain there are two pairs of cysteines known, in the growth hormone receptor, to be involved in disulfide bonds. +-------------------------------- -------xxxxxxx--------------------------+ | C C C C Extracellular XXXXXXX Cytoplasmic | +- |-|-------|--|------------------------xxxxxxx--------------------------+ | | | | Transmembrane +-+ +-- + Two patterns to detect this family of receptors were used. The first one is derived from the first N-terminal disulfide loop, the second is a tryptophan-rich pattern located at the C-terminal extremity of the extracellular region.

Consensus pattern: C-[LVFYR]-x(7,8)-[STIVDN]-C-x-W [The two C's are linked by a disulfide bond]-

Consensus pattern: [STGL]-x-W-[SG]-x-W-S-

[1] Bazan J.F. Biochem. Biophys. Res. Commun. 164:788-795(1989).

[2] Bazan J.F. Proc. Natl. Acad. Sci. U.S.A. 87:6934-6938(1990).

[3] Cosman D., Lyman S.D., Idzerda R.L., Beckmann M.P., Park L.S., Goodwin R.G., March C.J. Trends Biochem. Sci. 15:265-270(1990).

[4] d'Andrea A.D., Fasman G.D., Lodish H.F. Cell 58:1023-1024(1989).

[5] d'Andrea A.D., Fasman G.D., Lodish H.F. Curr. Opin. Cell Biol. 2:648-651(1990).

210. Phosphoribosylglycinamide formyltransferase active site (formyl_transf) Phosphoribosylglycinamide formyltransferase (EC 2.1.2.2) (GART) [1] catalyzes the third step in de novo purine biosynthesis, the transfer of a formyl group to 5'-phosphoribosylglycinamide. In higher eukaryotes, GART is part of a multifunctional enzyme polypeptide that catalyzes three of the steps of purine biosynthesis. In bacteria, plants and yeast, GART is a monofunctional protein of about 200 amino-acid residues. In the Escherichia coli enzyme, an aspartic acid residue has been shown to be involved in the catalytic mechanism. The region around this active site residue is well conserved in GART from prokaryotic and eukaryotic sources and can be used as a signature pattern. Mammalian

formyltetrahydrofolate dehydrogenase (EC 1.5.1.6) [2] is a cytosolicenzyme responsible for the NADP-dependent decarboxylative reduction of 10-formyltetrahydrofolate into tetrahydrofolate. It is a protein of about 900 amino acids consisting of three domains; the N-terminal domain (200 residues) is structurally related to GARTs.Escherichia coli methionyl-tRNA formyltransferase (EC 2.1.2.9) (gene fmt) [3]is the enzyme responsible for modifying the free amino group of the aminoacylmoiety of methionyl-□A(fMet). The central part of fmt seems to be evolutionary related to GART's active site region.

Consensus pattern: G-x-[STM]-[IVT]-x-[FYWVQ]-[VMAT]-x-[DEVM]-x-[LIVMY]-D-x-G- x(2)-[LIVT]-x(6)-[LIVM] [D is the active site residue] -

[1] Inglese J., Smith J.M., Benkovic S.J. Biochemistry 29:6678-6687(1990).
[2] Cook R.J., Lloyd R.S., Wagner C. J. Biol. Chem. 266:4965-4973(1991).
[3] Guillon J.-M., Mechulam Y., Schmitter J.-M., Blanquet S., Fayat G. J. Bacteriol. 174:4294-4301(1992).

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211. G10 protein signatures

A Xenopus protein known as G10 [1] has been found to be highly conserved in a wide range of eukaryotic species. The function of G10 is still unknown. G10 is a protein of about 17 to 18 Kd (143 to 157 residues) which is hydrophilic and whose C-terminal half is rich in cysteines and could be involved in metal-binding. As signature patterns, two of these cysteine-rich segments were selected.

Consensus pattern: L-C-C-x-[KR]-C-x(4)-[DE]-x-N-x(4)-C-x-C-R-V-P-
Consensus pattern: C-x-H-C-G-C-[KRH]-G-C-[SA]-

[1] McGrew L.L., Dworkin-Rastl E., Dworkin M.B., Richter J.D. Genes Dev. 3:803-815(1989).

212. G-protein alpha subunit

G proteins couple receptors of extracellular signals to intracellular signaling pathways. The G protein alpha subunit binds guanyl nucleotide and is a weak GTPase.
Number of members: 195

[1] Coleman DE, Berghuis AM, Lee E, Linder ME, Gilman AG, Sprang SR, Science 1994;265:1405-1412.
[2] How G proteins work: a continuing story. Coleman DE, Sprang SR, Trends Biochem Sci 1996;21:41-44.

213. Glucose-6-phosphate dehydrogenase active site (G6PD)

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (G6PD) [1] catalyzes the first step in the pentose pathway, the reduction of glucose-6-phosphate to gluconolactone 6-phosphate. A lysine residue has been identified as are active nucleophile associated with the activity of the enzyme. The sequence around this lysine is totally conserved from bacterial to mammalian G6PD's and can be used as a signature pattern

Consensus pattern: D-H-Y-L-G-K-[EQK] [K is the active site residue]-

[1] Jeffery J., Persson B., Wood I., Bergman T., Jeffery R., Joernvall H. Eur. J. Biochem. 212:41-49(1993).

214. GATA-type zinc finger domain

The GATA family of transcription factors are proteins that bind to DNA sites with the consensus sequence (A/T)GATA(A/G), found within the regulatory region of a number of genes. Proteins currently known to belong to this family are: - GATA-1 [1] (also known as Eryf1, GF-1 or NF-E1), which binds to the GATA region of globin genes and other genes expressed in erythroid cells. It is a transcriptional activator which probably serves as a general 'switch' factor for erythroid development. - GATA-2 [2], a transcriptional activator which regulates endothelin-1 gene expression in endothelial cells. - GATA-3 [3], a transcriptional activator which binds to the enhancer of the T-cell receptor alpha and delta genes. - GATA-4 [4], a transcriptional activator expressed in endodermally derived tissues and heart. - Drosophila protein pannier (or DGATAa) (gene pnr) which acts as a repressor of the achaete-scute complex (as-c). - Bombyx mori BCFI [5], which regulates the expression of chorion genes. - Caenorhabditis elegans elt-1 and elt-2, transcriptional activators of genes containing the GATA region, including vitellogenin genes [6]. - Ustilago maydis urbs1 [7], a protein involved in the repression of the biosynthesis of siderophores. - Fission yeast protein GAF2.All these transcription factors contain a pair of highly similar 'zinc finger' type domains with the consensus sequence C-x2-C-x17-C-x2-C.Some other proteins contain a single zinc finger motif highly related to those of the GATA transcription factors. These proteins are: - Drosophila box A-binding factor (ABF) (also known as protein serpent (gene srp)) which may function as a transcriptional activator protein and may play a key role in the organogenesis of the fat body. - Emericella nidulans areA [8], a transcriptional activator which mediates nitrogen metabolite repression. - Neurospora crassa nit-2 [9], a transcriptional activator which turns on the expression of genes coding for enzymes required for the use of a variety of secondary nitrogen sources, during conditions of nitrogen limitation. - Neurospora crassa white collar proteins 1 and 2 (WC-1 and WC-2), which control expression of light-regulated genes. - Saccharomyces cerevisiae DAL81 (or UGA43), a negative nitrogen regulatory protein. - Saccharomyces cerevisiae GLN3, a positive nitrogen regulatory protein. - Saccharomyces cerevisiae GAT1. - Saccharomyces cerevisiae GZF3.

Consensus pattern: C-x-[DN]-C-x(4,5)-[ST]-x(2)-W-[HR]-[RK]-x(3)-[GN]-x(3,4)- C-N-[AS]-C [The four C's are zinc ligands]

[1] Trainor C.D., Evans T., Felsenfeld G., Boguski M.S. Nature 343:92-96(1990).

[2] Lee M.E., Temizer D.T., Clifford J.A., Quertermous T. J. Biol. Chem. 266:16188-16192(1991).

[3] Ho I.-C., Vorhees P., Marin N., Oakley B.K., Tsai S.-F., Orkin S.H., Leiden J.M. EMBO J. 10:1187-1192(1991).

[4] Spieth J., Shim Y.H., Lea K., Conrad R., Blumenthal T. Mol. Cell. Biol. 11:4651-4659(1991).

[5] Drevet J.R., Skeiky Y.A., Iatrou K. J. Biol. Chem. 269:10660-10667(1994).

[6] Hawkins M.G., McGhee J.D. J. Biol. Chem. 270:14666-14671(1995).

[7] Voisard C.P.O., Wang J., Xu P., Leong S.A., McEvoy J.L. Mol. Cell. Biol. 13:7091-7100(1993).

[8] Arst H.N. Jr., Kudla B., Martinez-Rossi N.M., Caddick M.X., Sibley S., Davies R.W. Trends Genet. 5:291-291(1989).
[9] Fu Y.-H., Marzluf G.A. Mol. Cell. Biol. 10:1056-1065(1990).

215. Glutamine amidotransferases class-I active site (GATase)

A large group of biosynthetic enzymes are able to catalyze the removal of the ammonia group from glutamine and then to transfer this group to a substrate to form a new carbon-nitrogen group. This catalytic activity is known asglutamine amidotransferase (GATase) (EC 2.4.2.-) [1]. The GATase domain exists either as a separate polypeptidic subunit or as part of a larger polypeptide fused in different ways to a synthase domain. On the basis of sequence similarities two classes of GATase domains have been identified [2,3]: class-I(also known as trpG-type) and class-II (also known as purF-type). Class-I GATase domains have been found in the following enzymes: - The second component of anthranilate synthase (AS) (EC 4.1.3.27) [4]. AS catalyzes the biosynthesis of anthranilate from chorismate and glutamine. AS is generally a dimeric enzyme: the first component can synthesize anthranilate using ammonia rather than glutamine, whereas component II provides the GATase activity. In some bacteria and in fungi the GATase component of AS is part of a multifunctional protein that also catalyzes other steps of the biosynthesis of tryptophan. - The second component of 4-amino-4-deoxychorismate (ADC) synthase (EC 4.1.3. -), a dimeric prokaryotic enzyme that function in the pathway that catalyzes the biosynthesis of para-aminobenzoate (PABA) from chorismate and glutamine. The second component (gene pabA) provides the GATase activity [4]. - CTP synthase (EC 6.3.4.2). CTP synthase catalyzes the final reaction in the biosynthesis of pyrimidine, the ATP-dependent formation of CTP from UTP and glutamine. CTP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the C-terminal section [2]. - GMP synthase (glutamine-hydrolyzing) (EC 6.3.5.2). GMP synthase catalyzes the ATP-dependent formation of GMP from xanthosine 5'-phosphate and glutamine. GMP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the N-terminal section [5]. - Glutamine-dependent carbamoyl-phosphate synthase (EC 6.3.5.5) (GD-CPSase); an enzyme involved in both arginine and pyrimidine biosynthesis and which catalyzes the ATP-dependent formation of carbamoyl phosphate from glutamine and carbon dioxide. In bacteria GD-CPSase is composed of two subunits: the large chain (gene carB) provides the CPSase activity, while the small chain (gene carA) provides the GATase activity. In yeast the enzyme involved in arginine biosynthesis is also composed of two subunits: CPA1 (GATase), and CPA2 (CPSase). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by a large multifunctional enzyme (called URA2 in yeast, rudimentary in Drosophila, and CAD in mammals). The GATase domain is located at the N-terminal extremity of this polyprotein [6]. - Phosphoribosylformylglycinamidine synthase II (EC 6.3.5.3), an enzyme that catalyzes the fourth step in the de novo biosynthesis of purines. In some species of bacteria, FGAM synthase II is composed of two subunits: a small chain (gene purQ) which provides the GATase activity and a large chain (gene purL) which provides the aminator activity. - The histidine amidotransferase hisH, an enzyme that catalyzes the fifth step in the biosynthesis of histidine in prokaryotes.In the second component of AS a cysteine has been shown [7] to be essentialfor the amidotransferase activity. The sequence around this residue is well conserved in all the above GATase domains and can be used as a signature pattern for class-I GATase.-

Consensus pattern: [PAS]-[LIVMFYT]-[LIVMFY]-G-[LIVMFY]-C-[LIVMFYN]-G-x-[QEH]- x-[LIVMFA] [C is the active site residue]-

[1] Buchanan J.M. Adv. Enzymol. 39:91-183(1973).
[2] Weng M., Zalkin H. J. Bacteriol. 169:3023-3028(1987).
[3] Nyunoya H., Lusty C.J. J. Biol. Chem. 259:9790-9798(1984).
[4] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989).
[5] Zalkin H., Argos P., Narayana S.V.L., Tiedeman A.A., Smith J.M. J. Biol. Chem. 260:3350-3354(1985).
[6] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).
[7] Tso J.Y., Hermodson M.A., Zalkin H. J. Biol. Chem. 255:1451-1457(1980).

216. Glutamine amidotransferases class-II active site (GATase_2)

A large group of biosynthetic enzymes are able to catalyze the removal of the ammonia group from glutamine and then to transfer this group to a substrate to form a new carbon-nitrogen group. This catalytic activity is known as glutamine amidotransferase (GATase) (EC 2.4.2.-) [1]. The GATase domain exists either as a separate polypeptidic subunit or as part of a larger polypeptide fused in different ways to a synthase domain. On the basis of sequence similarities two classes of GATase domains have been identified [2,3]: class-I(also known as trpG-type) and class-II (also known as purF-type). Class-II GATase domains have been found in the following enzymes: - Amido phosphoribosyltransferase (glutamine phosphoribosylpyrophosphate amidotransferase) (EC 2.4.2.14). An enzyme which catalyzes the first step in purine biosynthesis, the transfer of the ammonia group of glutamine to PRPP to form 5-phosphoribosylamine (gene purF in bacteria, ADE4 in yeast). - Glucosamine--fructose-6-phosphate aminotransferase (EC 2.6.1.16). This enzyme catalyzes a key reaction in amino sugar synthesis, the formation of glucosamine 6-phosphate from fructose 6-phosphate and glutamine (gene glmS in Escherichia coli, nodM in Rhizobium, GFA1 in yeast) - Asparagine synthetase (glutamine-hydrolyzing) (EC 6.3.5.4). This enzyme is responsible for the synthesis of asparagine from aspartate and glutamine. A cysteine is present at the N-terminal extremity of the mature form of all these enzymes. The cysteine has been shown, in amido phosphoribosyltransferase [4] and in asparagine synthetase [5] to be important for the catalytic mechanism.

Consensus pattern: <x(0,11)-C-[GS]-[IV]-[LIVMFYW]-[AG] [C is the active site residue]-

[1] Buchanan J.M. Adv. Enzymol. 39:91-183(1973).
[2] Weng M., Zalkin H. J. Bacteriol. 169:3023-3028(1987).
[3] Nyunoya H., Lusty C.J. J. Biol. Chem. 259:9790-9798(1984).
[4] van Heeke G., Schuster M. J. Biol. Chem. 264:5503-5509(1989).
[5] Vollmer S.J., Switzer R.L., Hermodson M.A., Bower S.G., Zalkin H. J. Biol. Chem. 258:10582-10585(1983).

217. GDP dissociation inhibitor (GDI)

[1] Schalk I, Zeng K, Wu SK, Stura EA, Matteson J, Huang M, Tandon A, Wilson IA, Balch WE, Nature 1996;381:42-48.

218. Oxidoreductase family (GFO_IDH_MocA)

This family of enzymes utilise NADP or NAD. This family: is called the GFO/IDH/MOCA family in swiss-prot.

[1] Kingston RL, Scopes RK, Baker EN, Structure 1996;4:1413-1428.

219. GHMP kinases putative ATP-binding domain

The following kinases contains, in their N-terminal section, a conserved Gly/Ser-rich region which is probably involved in the binding of ATP [1]. These kinases are listed below. - Galactokinase (EC 2.7.1.6). - Homoserine kinase (EC 2.7.1.39). - Mevalonate kinase (EC 2.7.1.36). - Phosphomevalonate kinase (EC 2.7.4.2). This group of kinases was called 'GHMP' (from the first letter of their substrate)

Consensus pattern: [LIVM]-[PK]-x-[GSTA]-x(0,1)-G-L-[GS]-S-S-[GSA]-[GSTAC]-

[1] Tsay Y.H., Robinson G.W. Mol. Cell. Biol. 11:620-631(1991).

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220. Glucose inhibited division protein A family signatures (GIDA)

Bacterial glucose inhibited division protein A (gene gidA) is a protein of 70Kd whose function is not yet known and whose sequence is highly conserved. It is evolutionary related to yeast hypothetical protein YGL236C, Caenorhabditis elegans hypothetical protein F52H3.2 and a Bacillus subtilis protein called gid (and which is different from B.subtilis gidA). Two highly conserved regions were selected as signature patterns. Both regions are located in the central region of the protein.

Consensus pattern: [GS]-[PT]-x-Y-C-P-S-[LIVM]-E-x-K-[LIVM]-x-[KR]-

Consensus pattern: A-G-Q-x-[NT]-G-x(2)-G-Y-x-E-[SAG](3)-[QS]-G-[LIVM](2)-A-G-[LIVMT]-N-A-

221. (GLFV_dehydrog)

Glu / Leu / Phe / Val dehydrogenases active site

- Glutamate dehydrogenases (EC 1.4.1.2, EC 1.4.1.3, and EC 1.4.1.4) (GluDH) are enzymes that catalyze the NAD- or NADP-dependent reversible deamination of glutamate into alpha-ketoglutarate [1,2]. GluDH isozymes are generally involved with either ammonia assimilation or glutamate catabolism.
- Leucine dehydrogenase (EC 1.4.1.9) (LeuDH) is a NAD-dependent enzyme that catalyzes the reversible deamination of leucine and several other aliphatic amino acids to their keto analogues [3].
- Phenylalanine dehydrogenase (EC 1.4.1.20) (PheDH) is a NAD-dependent enzyme that catalyzes the reversible deamidation of L-phenylalanine into phenyl-pyruvate [4].
- Valine dehydrogenase (EC 1.4.1.8) (ValDH) is a NADP-dependent enzyme that catalyzes the reversible deamidation of L-valine into 3-methyl-2-oxobutanoate [5].

These dehydrogenases are structurally and functionally related. A conserved lysine residue located in a glycine-rich region has been implicated in the catalytic mechanism. The conservation of the region around this residue allows the derivation of a signature pattern for such type of enzymes.

Consensus pattern[LIV]-x(2)-G-G-[SAG]-K-x-[GV]-x(3)-[DNST]-[PL] [K is the active site residue] Sequences known to belong to this class detected by the pattern ALL.

Note all known sequences from this family have Pro in the last position of the pattern with the exception of yeast GluDH which as Leu.

[1] Britton K.L., Baker P.J., Rice D.W., Stillman T.J. Eur. J. Biochem. 209:851-859(1992).
[2] Benachenhou-Lahfa N., Forterre P., Labedan B. J. Mol. Evol. 36:335-346(1993).
[3] Nagata S., Tanizawa K., Esaki N., Sakamoto Y., Ohshima T., Tanaka H., Soda K. Biochemistry 27:9056-9062(1988).
[4] Takada H., Yoshimura T., Ohshima T., Esaki N., Soda K. J. Biochem. 109:371-376(1991).
[5] Hutchinson C.R., Tang L. J. Bacteriol. 175:4176-4185(1993).

222. GMC oxidoreductases signatures

The following FAD flavoproteins oxidoreductases have been found [1,2] to be evolutionary related. These enzymes, which are called 'GMC oxidoreductases', are listed below. - Glucose oxidase (EC 1.1.3.4) (GOX) from Aspergillus niger. Reaction catalyzed: glucose + oxygen -> delta-gluconolactone + hydrogen peroxide. - Methanol oxidase (EC 1.1.3.13) (MOX) from fungi. Reaction catalyzed: methanol + oxygen -> acetaldehyde + hydrogen peroxide. - Choline dehydrogenase (EC 1.1.99.1) (CHD) from bacteria. Reaction catalyzed: choline + unknown acceptor -> betaine acetaldehyde + reduced acceptor. - Glucose dehydrogenase (GLD) (EC 1.1.99.10) from Drosophila. Reaction catalyzed: glucose + unknown acceptor -> delta-gluconolactone + reduced acceptor. - Cholesterol oxidase (CHOD) (EC 1.1.3.6) from Brevibacterium sterolicum and Streptomyces strain SA-COO. Reaction catalyzed: cholesterol + oxygen -> cholest-4-en-3-one + hydrogen peroxide. - AlkJ [3], an alcohol dehydrogenase from Pseudomonas oleovorans, which converts aliphatic medium-chain-length alcohols into aldehydes. This family also includes a lyase: - (R)-mandelonitrile lyase (EC 4.1.2.10) (hydroxynitrile lyase) from plants [4], an enzyme involved in cyanogenis, the release of hydrogen cyanide from injured tissues. These enzymes are proteins of size ranging from 556 (CHD) to 664 (MOX) amino acid residues which share a number of regions of sequence similarities. One of these regions, located in the N-terminal section, corresponds to the FAD ADP-binding domain. The function of the other conserved domains is not yet known; two of these domains were selected as signature patterns. The first one is located in the N-terminal section of these enzymes, about 50 residues after the ADP-binding domain, while the second one is located in the central section.

Consensus pattern: [GA]-[RKN]-x-[LIV]-G(2)-[GST](2)-x-[LIVM]-N-x(3)-[FYWA]- x(2)-[PAG]-x(5)-[DNESH]-

Consensus pattern: [GS]-[PSTA]-x(2)-[ST]-P-x-[LIVM](2)-x(2)-S-G-[LIVM]-G-

[1] Cavener D.R. J. Mol. Biol. 223:811-814(1992).
[2] Henikoff S., Henikoff J.G. Genomics 19:97-107(1994).
[3] van Beilen J.B., Eggink G., Enequist H., Bos R., Witholt B. Mol. Microbiol. 6:3121-3136(1992).
[4] Cheng I.P., Poulton J.E. Plant Cell Physiol. 34:1139-1143(1993).

223. (GMP_synt_C)

Glutamine amidotransferases class-I active site

A large group of biosynthetic enzymes are able to catalyze the removal of the ammonia group from glutamine and then to transfer this group to a substrate to form a new carbon-nitrogen group. This catalytic activity is known as glutamine amidotransferase (GATase) (EC 2.4.2.-) [1]. The GATase domain exists either as a separate polypeptidic subunit or as part of a larger polypeptide fused in different ways to a synthase domain. On the basis of sequence similarities two classes of GATase domains have been identified [2,3]: class-I (also known as trpG-type) and class-II (also known as purF-type). Class-I GATase domains have been found in the following enzymes:

- The second component of anthranilate synthase (AS) (EC 4.1.3.27) [4]. AS catalyzes the biosynthesis of anthranilate from chorismate and glutamine. AS is generally a dimeric enzyme: the first component can synthesize anthranilate using ammonia rather than glutamine, whereas component II provides the GATase activity. In some bacteria and in fungi the GATase component of AS is part of a multifunctional protein that also catalyzes other steps of the biosynthesis of tryptophan.
- The second component of 4-amino-4-deoxychorismate (ADC) synthase (EC 4.1.3. -), a dimeric prokaryotic enzyme that function in the pathway that catalyzes the biosynthesis of para-aminobenzoate (PABA) from chorismate and glutamine. The second component (gene pabA) provides the GATase activity [4].
- CTP synthase (EC 6.3.4.2). CTP synthase catalyzes the final reaction in the biosynthesis of pyrimidine, the ATP-dependent formation of CTP from UTP and glutamine. CTP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the C-terminal section [2].
- GMP synthase (glutamine-hydrolyzing) (EC 6.3.5.2). GMP synthase catalyzes the ATP-dependent formation of GMP from xanthosine 5'-phosphate and glutamine. GMP synthase is a single chain enzyme that contains two distinct domains; the GATase domain is in the N-terminal section [5].

- Glutamine-dependent carbamoyl-phosphate synthase (EC 6.3.5.5) (GD-CPSase); an enzyme involved in both arginine and pyrimidine biosynthesis and which catalyzes the ATP-dependent formation of carbamoyl phosphate from glutamine and carbon dioxide. In bacteria GD-CPSase is composed of two subunits: the large chain (gene carB) provides the CPSase activity, while the small chain (gene carA) provides the GATase activity. In yeast the enzyme involved in arginine biosynthesis is also composed of two subunits: CPA1 (GATase), and CPA2 (CPSase). In most eukaryotes, the first three steps of pyrimidine biosynthesis are catalyzed by a large multifunctional enzyme (called URA2 in yeast, rudimentary in Drosophila, and CAD in mammals). The GATase domain is located at the N-terminal extremity of this polyprotein [6].
- Phosphoribosylformylglycinamidine synthase II (EC 6.3.5.3), an enzyme that catalyzes the fourth step in the de novo biosynthesis of purines. In some species of bacteria, FGAM synthase II is composed of two subunits: a small chain (gene purQ) which provides the GATase activity and a large chain (gene purL) which provides the aminator activity.
- The histidine amidotransferase hisH, an enzyme that catalyzes the fifth step in the biosynthesis of histidine in prokaryotes.

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In the second component of AS a cysteine has been shown [7] to be essential for the amidotransferase activity. The sequence around this residue is well conserved in all the above GATase domains and can be used as a signature pattern for class-I GATase.

Consensus pattern[PAS]-[LIVMFYT]-[LIVMFY]-G-[LIVMFY]-C-[LIVMFYN]-G-x-[QEH]- x-[LIVMFA] [C is the active site residue] Sequences known to belong to this class detected by the pattern ALL, except for 6 sequences.

Note: in the first position of the pattern Pro is found in all cases except in the slime mold GD-CPSase where it is replaced by Ala.

[1] Buchanan J.M. Adv. Enzymol. 39:91-183(1973).
[2] Weng M., Zalkin H. J. Bacteriol. 169:3023-3028(1987).
[3] Nyunoya H., Lusty C.J. J. Biol. Chem. 259:9790-9798(1984).
[4] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989).

[5] Zalkin H., Argos P., Narayana S.V.L., Tiedeman A.A., Smith J.M. J. Biol. Chem. 260:3350-3354(1985).

[6] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).

[7] Tso J.Y., Hermodson M.A., Zalkin H. J. Biol. Chem. 255:1451-1457(1980).

224. Glutathione peroxidases signatures (GSHPx)

Glutathione peroxidase (EC 1.11.1.9) (GSHPx) [1,2] is an enzyme that catalyzes the reduction of hydroxyperoxides by glutathione. Its main function is to protect against the damaging effect of endogenously formed hydroxyperoxides. In higher vertebrates at least four forms of GSHPx are known to exist: a ubiquitous cytosolic form (GSHPx-1), a gastrointestinal cytosolic for (GSHPx-GI) [3], a plasma secreted form (GSHPx-P) [4], and a epididymal secretory form (GSHPx-EP). In addition to these characterized forms, the sequence of a protein of unknown function [5] has been shown to be evolutionary related to those of GSHPx's. In filarial nematode parasites such as Brugia pahangi the major soluble cuticular protein, known as gp29, is a secreted GSHPx which could provide a mechanism of resistance to the immune reaction of the mammalian host by neutralizing the products of the oxidative burst of leukocytes [6].Escherichia coli protein btuE, a periplasmic protein involved in the transport of vitamin B12, is also evolutionary related to GSHPx's; the significance of this relationship is not yet clear. Selenium, in the form of selenocysteine [7] is part of the catalytic site of GSHPx. The sequence around the selenocysteine residue is moderately well conserved in GSHPx's and the related proteins and can be used as a signature pattern. As a second signature for this family of proteins a highly conserved octapeptide located in the central section of these proteins was selected.

Consensus pattern: [GN]-[RKHNFYC]-x-[LIVMFC]-[LIVMF](2)-x-N-[VT]-x-[STC]-x-C-[GA]-x-T [C is the active site selenocysteine residue]

Consensus pattern: [LIV]-[AGD]-F-P-[CS]-[NG]-Q-

[1] Mannervik B. Meth. Enzymol. 113:490-495(1985).

[2] Mullenbach G.T., Tabrizi A., Irvine B.D., Bell G.I., Tainer J.A., Hallewell R.A. Protein Eng. 2:239-246(1988).

[3] Chu F.F., Doroshow J.H., Esworthy R.S. J. Biol. Chem. 268:2571-2576(1993).

[4] Takahashi K., Akasaka M., Yamamoto Y., Kobayashi C., Mizoguchi J., Koyama J. J. Biochem. 108:145-148(1990).

[5] Dunn D.K., Howells D.D., Richardson J., Goldfarb P.S. Nucleic Acids Res. 17:6390-6390(1989).

[6] Cookson E., Blaxter M.L., Selkirk M.E. Proc. Natl. Acad. Sci. U.S.A. 89:5837-5841(1992).

[7] Stadtman T.C. Annu. Rev. Biochem. 59:111-127(1990).

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225. (GST)

Glutathione S-transferases

Function: conjugation of reduced glutathione to a variety of targets. Also included in the alignment, but are not GSTs S-crystallins from squid. Similarity to GST was previously noted. Eukaryotic elongation factors 1-gamma. Not known to have GST activity; similarity not previously recognized. Supported by HMM and manual alignment inspection. HSP26 family of stress-related proteins. including auxin-regulated proteins in plants and stringent starvation proteins in E. coli. Not known to have GST activity. Similarity not previously recognized. Supported by HMM and manual alignment inspection. Alignment spans entire protein.

226. GTP1/OBG family signature

A widespread family of GTP-binding proteins has been recently characterized [1,2]. This family currently includes: - Mouse and Xenopus protein DRG. - Human protein DRG2. - Drosophila protein 128up. - Fission yeast protein gtp1. - A Halobacterium cutirubrum hypothetical protein in a ribosomal protein gene cluster. - Bacillus subtilis protein obg. Obg has been experimentally shown to bind GTP. - Escherichia coli hypothetical protein yhbZ. - Haemophilus influenzae hypothetical protein HI0877. - Mycoplasma genitalium hypothetical protein MG384. - Yeast hypothetical protein YAL036c (FUN11). - Yeast hypothetical protein YGR173w. - Caenorhabditis elegans hypothetical protein C02F5.3.The function of the proteins that belong to this family is not yet known. They are polypeptides of about 40 to

48 Kd which contain the five small sequence elements characteristic of GTP-binding proteins [3]. As a signature pattern the region that correspond to the ATP/GTP B motif (also called G-3 inGTP-binding proteins) was selected.

Consensus pattern: D-[LIVM]-P-G-[LIVM](2)-[DEY]-[GN]-A-x(2)-G-x-G -

[1] Sazuka T., Tomooka Y., Ikawa Y., Noda M., Kumar S. Biochem. Biophys. Res. Commun. 189:363-370(1992).
[2] Hudson J.D., Young P.G. Gene 125:191-193(1993).
[3] Bourne H.R., Sanders D.A., McCormick F. Nature 349:117-127(1991).

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227. (GTP_EFTU1)
ATP/GTP-binding site motif A (P-loop)

From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix. This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5].There are numerous ATP- or GTP-binding proteins in which the P-loop is found. Listed below are a number of protein families for which the relevance of the presence of such motif has been noted: - ATP synthase alpha and beta subunits (see <PDOC00137>). - Myosin heavy chains. - Kinesin heavy chains and kinesin-like proteins (see <PDOC00343>). - Dynamins and dynamin-like proteins (see <PDOC00362>). - Guanylate kinase (see <PDOC00670>). - Thymidine kinase (see <PDOC00524>). - Thymidylate kinase (see <PDOC01034>). - Shikimate kinase (see <PDOC00868>). - Nitrogenase iron protein family (nifH/frxC) (see <PDOC00580>). - ATP-binding proteins involved in 'active transport' (ABC transporters) [7] (see <PDOC00185>). - DNA and RNA helicases [8,9,10]. - GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.). - Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.). - Nuclear protein ran (see <PDOC00859>). - ADP-ribosylation factors family (see <PDOC00781>). - Bacterial dnaA protein (see <PDOC00771>). - Bacterial recA protein (see <PDOC00131>). - Bacterial recF protein (see <PDOC00539>). - Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.). - DNA mismatch repair proteins mutS family (See <PDOC00388>). - Bacterial type II secretion system protein E (see <PDOC00567>).Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the glycolytic kinases. In other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved for adenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.

-Consensus pattern: [AG]-x(4)-G-K-[ST]-

[1] Walker J.E., Saraste M., Runswick M.J., Gay N.J. EMBO J. 1:945-951(1982).
[2] Moller W., Amons R. FEBS Lett. 186:1-7(1985).
[3] Fry D.C., Kuby S.A., Mildvan A.S. Proc. Natl. Acad. Sci. U.S.A. 83:907-911(1986).
[4] Dever T.E., Glynias M.J., Merrick W.C. Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).
[5] Saraste M., Sibbald P.R., Wittinghofer A. Trends Biochem. Sci. 15:430-434(1990).
[6] Koonin E.V. J. Mol. Biol. 229:1165-1174(1993).
[7] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg. Biomembr. 22:571-592(1990).
[8] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).
[9] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).
[10] Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M. Nucleic Acids Res. 17:4713-4730(1989).

GTP-binding elongation factors signature (GTP_EFTU2)

Elongation factors [1,2] are proteins catalyzing the elongation of peptide chains in protein biosynthesis. In both prokaryotes and eukaryotes, there are three distinct types of elongation factors, as described in the following table: --- Eukaryotes Prokaryotes Function --- EF-1alpha EF-Tu Binds GTP and an aminoacyl-tRNA; delivers the latter to

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the A site of ribosomes. EF-1beta EF-Ts Interacts with EF-1a/EF-Tu to displace GDP and thus allows the regeneration of GTP-EF-1a. EF-2 EF-G Binds GTP and peptidyl-tRNA and translocates the latter from the A site to the P site. ---The GTP-binding elongation factor family also includes the following proteins: - Eukaryotic peptide chain release factor GTP-binding subunits [3]. These proteins interact with release factors that bind to ribosomes that have encountered a stop codon at their decoding site and help them to induce release of the nascent polypeptide. The yeast protein was known as SUP2 (and also as SUP35, SUF12 or GST1) and the human homolog as GST1-Hs. - Prokaryotic peptide chain release factor 3 (RF-3) (gene prfC). RF-3 is a class-II RF, a GTP-binding protein that interacts with class I RFs (see <PDOC00607>) and enhance their activity [4]. - Prokaryotic GTP-binding protein lepA and its homolog in yeast (gene GUF1) and in Caenorhabditis elegans (ZK1236.1). - Yeast HBS1 [5]. - Rat statin S1 [6], a protein of unknown function which is highly similar to EF-1alpha. - Prokaryotic selenocysteine-specific elongation factor selB [7], which seems to replace EF-Tu for the insertion of selenocysteine directed by the UGA codon. - The tetracycline resistance proteins tetM/tetO [8,9] from various bacteria such as Campylobacter jejuni, Enterococcus faecalis, Streptococcus mutans and Ureaplasma urealyticum. Tetracycline binds to the prokaryotic ribosomal 30S subunit and inhibits binding of aminoacyl-tRNAs. These proteins abolish the inhibitory effect of tetracycline on protein synthesis. - Rhizobium nodulation protein nodQ [10]. - Escherichia coli hypothetical protein yihK [11].In EF-1-alpha, a specific region has been shown [12] to be involved in a conformational change mediated by the hydrolysis of GTP to GDP. This region is conserved in both EF-1alpha/EF-Tu as well as EF-2/EF-G and thus seems typical for GTP-dependent proteins which bind non-initiator tRNAs to the ribosome. The pattern developed for this family of proteins include that conserved region.

Consensus pattern: D-[KRSTGANQFYW]-x(3)-E-[KRAQ]-x-[RKQD]-[GC]-[IVMK]-[ST]-[IV]-x(2)-[GSTACKRNQ]-

[1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).

[2] Moldave K. Annu. Rev. Biochem. 54:1109-1149(1985).

[3] Stansfield I., Jones K.M., Kushnirov V.V., Dagkesamanskaya A.R., Poznyakovski A.I., Paushkin S.V., Nierras C.R., Cox B.S., Ter-Avanesyan M.D., Tuite M.F. EMBO J. 14:4365-4373(1995).

[4] Grentzmann G., Brechemier-Baey D., Heurgue-Hamard V., Buckingham R.H. J. Biol. Chem. 270:10595-10600(1995).

[5] Nelson R.J., Ziegelhoffer T., Nicolet C., Werner-Washburne M., Craig E.A. Cell 71:97-105(1992).

[6] Ann D.K., Moutsatsos I.K., Nakamura T., Lin H.H., Mao P.-L., Lee M.-J., Chin S., Liem R.K.H., Wang E. J. Biol. Chem. 266:10429-10437(1991).

[7] Forchammer K., Leinfeldr W., Bock A. Nature 342:453-456(1989).

[8] Manavathu E.K., Hiratsuka K., Taylor D.E. Gene 62:17-26(1988).

[9] Leblanc D.J., Lee L.N., Titmas B.M., Smith C.J., Tenover F.C. J. Bacteriol. 170:3618-3626(1988).

[10] Cervantes E., Sharma S.B., Maillet F., Vasse J., Truchet G., Rosenberg C. Mol. Microbiol. 3:745-755(1989).

[11] Plunkett G. III, Burland V.D., Daniels D.L., Blattner F.R. Nucleic Acids Res. 21:3391-3398(1993).

[12] Moller W., Schipper A., Amons R. Biochimie 69:983-989(1987).

228. GTP cyclohydrolase II.

GTP cyclohydrolase II catalyses the first committed step in the biosynthesis of riboflavin.

[1] Richter G, Ritz H, Katzenmeier G, Volk R, Kohnle A, Lottspeich F, Allendorf D, Bacher A, J Bacteriol 1993;175:4045-4051.

229. Galactose-1-phosphate uridyl transferase signatures (GalP_UDP_transf)

Galactose-1-phosphate uridyl transferase (EC 2.7.7.10) (galT) catalyzes the transfer of an uridyldiphosphate group on galactose (or glucose) 1-phosphate. During the reaction, the uridyl moiety links to a histidine residue. In the Escherichia coli enzyme, it has been shown [1] that two histidine residues separated by a single proline residue are essential for enzyme activity. On the basis of sequence similarities, two apparently unrelated families seem to exist. Class-I enzymes are found in eukaryotes as well as some bacteria such as Escherichia coli or Streptomyces lividans, while class-II enzymes have been found so far only in bacteria such as Bacillus subtilis or Lactobacillus helveticus [2]. Signature patterns for both families were developed. For class-I enzymes the signature is based on the active site residues. For class-II enzymes a region which also includes two conserved histidines was chosen.

Consensus pattern: F-E-N-[RK]-G-x(3)-G-x(4)-H-P-H-x-Q [The two H's are the active site residues]-

Consensus pattern: D-L-P-I-V-G-G-[ST]-[LIVM](2)-[SA]-H-[DEN]-H-[FY]-Q-G-G -

Note: class-I enzymes are structurally related to the HIT family of proteins (see <PDOC00694

[1] Reichardt J.K.V., Berg P. Nucleic Acids Res. 16:9017-9026(1988).
[2] Mollet B., Pilloud N. J. Bacteriol. 173:4464-4473(1991).

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230. Gamma-thionins family signature

The following small plant proteins are evolutionary related:

- Gamma-thionins from wheat endosperm (gamma-purothionins) and barley (gamma- hordothionins) which are toxic to animal cells and inhibit protein synthesis in cell free systems [1].
- A flower-specific thionin (FST) from tobacco [2].
- Antifungal proteins (AFP) from the seeds of Brassicaceae species such as radish, mustard, turnip and Arabidopsis thaliana [3].
- Inhibitors of insect alpha-amylases from sorghum [4].
- Probable protease inhibitor P322 from potato.
- A germination-related protein from cowpea [5].
- Anther-specific protein SF18 from sunflower [6]. SF18 is a protein that contains a gamma-thionin domain at its N-terminus and a proline-rich C- terminal domain.
- Soybean sulfur-rich protein SE60 [7].
- Vicia faba antibacterial peptides fabatin-1 and -2.

In their mature form, these proteins generally consist of about 45 to 50amino-acid residues. As shown in the following schematic representation, these peptides contain eight conserved cysteines involved in disulfide bonds.

```
+----------------------------------------+ | +-------------------+ | | | | |
xxCxxxxxxxxxxxCxxxxxCxxxCxxxxxxxxxCxxxxxxCxCxxxC ******************|***| | |
+---|----------------+ | +-------------------+
```

'C': conserved cysteine involved in a disulfide bond.

'*': position of the pattern.

Consensus pattern: [KRG]-x-C-x(3)-[SV]-x(2)-[FYWH]-x-[GF]-x-C-x(5)-C-x(3)-C [The four C's are involved in disulfide bonds]-

[1] Bruix M., Jimenez M.A., Santoro J., Gonzalez C., Colilla F.J., Mendez E., Rico M. Biochemistry 32:715-724(1993).

[2] Gu Q., Kawata E.E., Morse M.-J., Wu H.-M., Cheung A.Y. Mol. Gen. Genet. 234:89-96(1992).

[3] Terras F.R.G., Torrekens S., van Leuven F., Osborn R.W., Vanderleyden J., Cammue B.P.A., Broekaert W.F. FEBS Lett. 316:233-240(1993).

[4] Bloch C. Jr., Richardson M. FEBS Lett. 279:101-104(1991).

[5] Ishibashi N., Yamauchi D., Miniamikawa T. Plant Mol. Biol. 15:59-64(1990).

[7] Choi Y., Choi Y.D., Lee J.S. Plant Physiol. 101:699-700(1993).

231. Gelsolin. Gelsolin repeat. Number of members: 170

[1]Medline: 97433077. The crystal structure of plasma gelsolin: implications for actin severing, capping, and nucleation. Burtnick LD, Koepf EK, Grimes J, Jones EY, Stuart DI, McLaughlin PJ, Robinson RC; Cell 1997;90:661-670.

232. Germin family signature

Germins [1] are a family of homopentameric cereal glycoproteins expressed during germination which may play a role in altering the properties of cell walls during germinative growth. It has been shown that wheat and barleygermins act as oxalate oxidases (EC 1.2.3.4), an enzyme that catalyzes the oxidative degradation of oxalate to carbonate and hydrogen peroxide. Germins are highly similar to: - Germin-like proteins from various plants such as rape, violet or white mustard. - Slime mold spherulins 1a and 1b which are proteins that accumulate specifically during spherulation, a process induced by various forms of environmental stress which leads to encystment and dormancy. As a signature pattern the best conserved region was selected: a decapeptide located in the central section of these proteins.

Consensus pattern: G-x(4)-H-x-H-P-x-A-x-E-[LIVM]-

[1] Lane B.G. FASEB J. 8:294-301(1994).

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233. (GlutR)

Glutamyl-tRNA reductase signature

Delta-aminolevulinic acid (ALA) is the obligatory precursor for the synthesis of all tetrapyrroles including porphyrin derivatives such as chlorophyll and heme. ALA can be synthesized via two different pathways: the Shemin (or C4) pathway which involves the single step condensation of succinyl-CoA and glycine and which is catalyzed by ALA synthase (EC 2.3.1.37) and via the C5pathway from the five-carbon skeleton of glutamate. The C5 pathway operates in the chloroplast of plants and algae, in cyanobacteria, in some eubacteria and in archaebacteria.

The initial step in the C5 pathway is carried out by glutamyl-tRNA reductase (GluTR) [1] which catalyzes the NADP-dependent conversion of glutamate- tRNA(Glu) to glutamate-1-semialdehyde (GSA) with the concomitant release of tRNA(Glu) which can then be recharged with glutamate by glutamyl-tRNA synthetase.

GluTR is a protein of about 50 Kd (467 to 550 residues) which contains a few conserved region. The best conserved region is located in positions 99 to 122 in the sequence of known GluTR. This region seems important for the activity of the enzyme. We have developed a signature pattern from that conserved region.

Consensus patternH-[LIVM]-x(2)-[LIVM]-[GSTAC](3)-[LIVM]-[DEQ]-S-[LIVMA]-[LIVM](2)-[GF]-E-x-[EQR]-[IV]-[LIT]-[STAG]-Q-[LIVM]-[KR] Sequences known to belong to this class detected by the pattern ALL.

[1] Jahn D., Verkamp E., Soell D. Trends Biochem. Sci. 17:215-218(1992).

234. (Glycoprotease)

Glycoprotease family signature (aka Peptidase_M22)

Glycoprotease (GCP) (EC 3.4.24.57) [1], or o-syaloglycoprotein endopeptidase, is a metalloprotease secreted by Pasteurella haemolytica which specifically cleaves O-sialoglycoproteins such as glycophorin A. The sequence of GCP is highly similar to the following uncharacterized proteins:

- Escherichia coli hypothetical protein ygjD (ORF-X).
- Bacillus subtilis hypothetical protein ydiE.
- Mycobacterium leprae hypothetical protein U229E.
- Mycobacterium tuberculosis hypothetical protein MtCY78.10.
- Synechocystis strain PCC 6803 hypothetical protein slr0807.
- Methanococcus jannaschii hypothetical protein MJ1130.
- Haloarcula marismortui hypothetical protein in HSH 3' region.
- Yeast hypothetical protein YKR038c.
- Yeast hypothetical protein QRI7.

One of the conserved regions contains two conserved histidines. It is possible that this region is involved in coordinating a metal ion such as zinc.

Consensus pattern[KR]-[GSAT]-x(4)-[FYWLH]-[DQNGK]-x-P-x-[LIVMFY]-x(3)-H- x(2)-[AG]-H-[LIVM] Sequences known to belong to this class detected by the pattern ALL.

Note: these proteins belong to family M22 in the classification of peptidases [2,E1].

□

[1] Abdullah K.M., Lo R.Y.C., Mellors A. J. Bacteriol. 173:5597-5603(1991).

□

[2] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

235. (Glucosamine_iso)

Glucosamine/galactosamine-6-phosphate isomerases signature

Glucosamine-6-phosphate isomerase (EC 5.3.1.10) (or Glc-6-P deaminase) is the enzyme responsible for the conversion of glucosamine 6-phosphate into fructose6 phosphate [1]. It is the last specific step in the pathway for N-acetylglucosamine (GlcNAC) utilization in bacteria such as Escherichia coli (gene nagB) or in fungi such as Candida albicans (gene NAG1).Glc-6-P isomerase is evolutionary related to: - A putative Escherichia coli galactosamine-6-phosphate isomerase (gene agaI) [2]. - Escherichia coli hypothetical protein yieK. - Bacillus subtilis hypothetical protein ybfT. As a signature pattern a conserved region located in the central part of these enzymes was selected. This region contains a conserved histidine which has been shown [1], in nagB, to be important for the pyranose ring-opening step of the catalytic mechanism

Consensus pattern: [LIVM]-x(3)-G-x-[LIT]-x-[LIV]-x-[LIVM]-x-G-[LIVM]-G-x- [DEN]-G-H-

[1] Oliva G., Fontes M.R.M., Garratt R.C., Altamirano M.M., Calcagno M.L., Horjales E. Structure 3:1323-1332(1995).

[2] Reizer J., Ramseier T.M., Reizer A., Charbit A., Saier M.H. Jr. Microbiology 142:231-250(1996).

236. Pneumovirus attachment glycoprotein G (glycoprotein G)

This family includes attachment proteins from respiratory synctial virus. Glycoprotein G has not been shown to have any neuraminidase or hemagglutinin activity (Swiss-Prot). The amino terminus is thought to be cytoplasmic, and the carboxyl terminus extracellular. The extracellular region contains four completely conserved cysteine residues.

[1] Johnson PR, Spriggs MK, Olmsted RA, Collins PL, Proc Natl Acad Sci U S A 1987;84:5625-5629.

237. Glycosyl transferases group 1

Mutations in this domain of Swiss:P37287 lead to disease (Paroxysmal Nocturnal haemoglobinuria). Members of this family transfer activated sugars to a variety of substrates, including glycogen, Fructose-6-phosphate and lipopolysaccharides. Members of this family transfer UDP, ADP, GDP or CMP linked sugars. The eukaryotic glycogen synthases may be distant members of this family.

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238. Glycosyl transferases (Glycos_transf_2)

Diverse family, transferring sugar from UDP-glucose, UDP-N-acetyl-galactosamine, GDP-mannose or CDP-abequose, to a range of substrates including cellulose, dolichol phosphate and teichoic acids.

239. (Glucos_transf_3)

Thymidine and pyrimidine-nucleoside phosphorylases signature

Thymidine phosphorylase (EC 2.4.2.4) catalyzes the reversible phosphorolysis of thymidine, deoxyuridine and their analogues to their respective bases and 2-deoxyribose 1-phosphate. This enzyme regulates the availability of thymidine and is therefore essential to nucleic acid metabolism.

In Escherichia coli (gene deoA), the enzyme is a dimer of identical subunits of about 48 Kd [1]. In humans it was first identified as platelet-derived endothelial cell growth factor (PD-ECGF) [E1] before being recognized [2] as thymidine phosphorylase.

Bacterial pyrimidine-nucleoside phosphorylase (EC 2.4.2.2) (gene pdp) [3] is an enzyme evolutionary and structurally related to thymidine phosphorylase.

A a well conserved region of 19 residues located in the N-terminal part of these proteins signature pattern for these enzymes was selected.

Consensus patternS-[GS]-R-[GA]-[LIV]-x(2)-[TA]-[GA]-G-T-x-D-x-[LIV]-E Sequences known to belong to this class detected by the pattern ALL.

[1] Walter M.R., Cook W.J., Cole L.B., Short S.A., Koszalka G.W., Krenitsky T.A., Ealick S.E. J. Biol. Chem. 265:14016-14022(1990).
[2] Furukawa T., Yoshimura A., Sumizawa T., Haraguchi M., Akiyama S.-I., Fukui K., Yamada Y. Nature 356:668-668(1992).
[3] Saxild H.H., Andersen L.N., Hammer K. J. Bacteriol. 178:424-434(1996).

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240. Glycos_transf_4. Glycosyl transferase. Number of members: 44.

[1] Medline: 95252686. A family of UDP-GlcNAc/MurNAc: polyisoprenol-P GlcNAc/MurNAc-1-P transferases. Lehrman MA; Glycobiology 1994;4:768-771.

241. Glycosyl hydrolases family 15. 21 members.

242. Glycosyl hydrolases family 16 signature
It has been shown [1] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities: - Bacterial beta-1,3-1,4-glucanases, or lichenases, (EC 3.2.1.73) mainly from Bacillus but also from Clostridium thermocellum (gene licB), Fibrobacter succinogenes and Rhodothermus marinus (gene bglA). - Bacillus circulans beta-1,3-glucanase A1 (EC 3.2.1.39) (gene glcA). - Lamarinase (EC 3.2.1.6) from Clostridium thermocellum (gene lam1). - Streptomyces coelicolor agarase (EC 3.2.1.81) (gene dagA). - Alteromonas carrageenovora kappa-carrageenase (EC 3.2.1.83) (gene

cgkA).Two closely clustered conserved glutamates have been shown [2] to be involved in the catalytic activity of Bacillus licheniformis lichenase. The region was used that contains these residues as a signature pattern.

Consensus pattern: E-[LIV]-D-[LIV]-x(0,1)-E-x(2)-[GQ]-[KRNF]-x-[PSTA] [The two E's are active site residues]-

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Juncosa M., Pons J., Dot T., Querol E., Planas A. J. Biol. Chem. 269:14530-14535(1994).

243. Glycosyl hydrolases family 17 signature

It has been shown [1,2] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities: - Glucan endo-1,3-beta-glucosidases (EC 3.2.1.39) (endo-(1->3)-beta- glucanase) from various plants. This enzyme may be involved in the defense of plants against pathogens through its ability to degrade fungal cell wall polysaccharides. - Glucan 1,3-beta-glucosidase (EC 3.2.1.58) (exo-(1->3)-beta-glucanase) from yeast (gene BGL2). This enzyme may play a role in cell expansion during growth, in cell-cell fusion during mating, and in spore release during sporulation. - Lichenases (EC 3.2.1.73) (endo-(1->3,1->4)-beta-glucanase) from various plants. The best conserved region in the sequence of these enzymes is located in their central section. This region contains a conserved tryptophan residue which could be involved in the interaction with the glucan substrates [2] and it also contains a conserved glutamate which has been shown [3] to act as the nucleophile in the catalytic mechanism. this region was used as a signature pattern.

Consensus pattern: [LIVM]-x-[LIVMFYWA](3)-[STAG]-E-[STA]-G-W-P-[STN]-x-[SAGQ] [E is an active site residue]-

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Ori N., Sessa G., Lotan T., Himmelhoch S., Fluhr R. EMBO J. 9:3429-3436(1990).
[3] Varghese J.N., Garrett T.P.J., Colman P.M., Chen L., Hoj P.J., Fincher G.B. Proc. Natl. Acad. Sci. U.S.A. 91:2785-2789(1994).

244. Glyoxalase I signatures

Glyoxalase I (EC 4.4.1.5) (lactoylglutathione lyase) catalyzes the first step of the glyoxal pathway, the transformation of methylglyoxal and glutathioneinto S-lactoylglutathione which is then converted by glyoxalase II to lactic acid [1]. Glyoxalase I is an ubiquitous enzyme which binds one mole of zinc per subunit. The bacterial and yeast enzymes are monomeric while the mammalian one is homodimeric. The sequence of glyoxalase I is well conserved. In bacteria and mammals, the enzyme is a protein of about 130 to 180 residues while in fungi it is about twice longer. In these organisms the enzyme is built out of the tandem repeat of an homologous domain. Two signature patterns for this family were derived. The first one is located in the N-terminal region while the second one is located in the central section of the protein and contains a conserved histidine that could be implicated in the binding of the zinc atom.

Consensus pattern: [HQ]-[IVT]-x-[LIVFY]-x-[IV]-x(5)-[STA]-x(2)-F-[YM]-x(2,3)- [LMF]-G-[LMF]-

Consensus pattern: G-[NTKQ]-x(0,5)-[GA]-[LVFY]-[GH]-H-[IVF]-[CGA]-x-[STAGLE]-x(2)-[DNC]-

[1] Kim N.-S., Umezawa Y., Ohmura S., Kato S. J. Biol. Chem. 268:11217-11221(1993).

245. (Glypican)

Glypicans signature

Glypicans [1,2] are a family of heparan sulfate proteoglycans which are anchored to cell membranes by a glycosylphosphatidylinositol (GPI) linkage. Structurally, these proteins consist of three separate domains:

a) A signal sequence;

b) An extracellular domain of about 500 residues that contains 12 conserved cysteines probably involved in disulfide bonds and which also contains the sites of attachment of the heparan sulfate glycosaminoglycan side chains;

c) A C-terminal hydrophobic region which is post-translationally removed after formation of the GPI-anchor.

The proteins known to belong to this family are:

- Glypican 1 (GPC1).
- Glypican 2 (GPC2) or cerebroglycan.
- Glypican 3 (GPC3) or OCI-5. In man, defects in GPC3 are the cause of a X-linked genetic disease, Simpson-Galabi-Behmel syndrome (SGBS).
- K-glypican.
- Glypican 5 (GPC5).
- Drosophila protein dally.

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The signature pattern that was developed for glypicans is located in the central section of the extracellular domain and contains five of the conserved cysteines.

Consensus patternC-x(2)-C-x-G-[LIVM]-x(4)-P-C-x(2)-[FY]-C-x(2)-[LIVM]-x(2)- G-C [The C's are probably involved in a disulfide bonds] Sequences known to belong to this class detected by the pattern ALL, except for dally.

[1] Weksberg R., Squire J.A., Templeton D.M. Nat. Genet. 12:225-227(1996).
[2] Watanabe K., Yamada H., Yamaguchi Y. J. Cell Biol. 130:1207-1218(1995).

246. Granins signatures

Granins (chromogranins or secretogranins) [1] are a family of acidic proteins present in the secretory granules of a wide variety of endocrine and neuro-endocrine cells. The exact function(s) of these proteins is not yet known but they seem to be the precursors of biologically active peptides and/or they may act as helper proteins in the packaging of peptide hormones and neuropeptides. Three members of this family of proteins show some sequence similarities: - Chromogranin A (CGA) [2]. CGA is a protein of about 420 residues; it is the precursor of the peptide pancreastatin which strongly inhibits glucose- induced insulin release from the pancreas. - Secretogranin 1 (chromogranin B). A sulfated protein of about 600 residues. - Secretogranin 2 (chromogranin C). A sulfated protein of about 650 residues. Apart from their subcellular location and the abundance of acidic residues(Asp and Glu), these proteins do not share many structural similarities. Only one short region, located in the C-terminal section, is conserved in all these proteins. Chromogranins A and B share a region of high similarity in their N-terminal section; this region includes two cysteine residues involved in a disulfide bond

Consensus pattern: [DE]-[SN]-L-[SAN]-x(2)-[DE]-x-E-L-
Consensus pattern: C-[LIVM](2)-E-[LIVM](2)-S-[DN]-[STA]-L-x-K-x-S-x(3)- [LIVM]-[STA]-x-E-C [The two C's are linked by a disulfide bond]-

[1] Huttner W.B., Gerdes H.-H., Rosa P. Trends Biochem. Sci. 16:27-30(1991).
[2] Simon J.-P., Aunis D. Biochem. J. 262:1-13(1989).

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247. grpE protein signature

In prokaryotes the grpE protein [1] stimulates, jointly with dnaJ, the ATPase activity of the dnaK chaperone. It seems to accelerate the release of ADP from dnaK thus allowing dnaK to recycle more efficiently. GrpE is a protein of about 22 to 25 Kd. In yeast, an evolutionary related mitochondrial protein(gene GRPE) has been shown [2] to associate with the mitochondrial hsp70protein and to thus play a role in the import of proteins from the cytoplasm. As a signature pattern, the most conserved region of grpE was selected. It is located in the C-terminal section.

Consensus pattern: [FL]-[DN]-[PHEA]-x(2)-[HM]-x-A-[LIVMTN]-x(16,20)-G-[FY]- x(3)-[DEG]-x(2)-[LIVM]-[RI]-x-[SA]-x-V-x-[IV]-

[1] Georgopoulos C., Welch W. Annu. Rev. Cell Biol. 9:601-635(1993).
[2] Bolliger L., Deloche O., Glick B.S., Georgopoulos C., Jenoe P., Kronidou N., Horst M., Morishima N., Schatz G. EMBO J. 13:1998-2006(1994).

248. Guanylate kinase signature and profile

Guanylate kinase (EC 2.7.4.8) (GK) [1] catalyzes the ATP-dependent phosphorylation of GMP into GDP. It is essential for recycling GMP and indirectly, cGMP. In prokaryotes (such as Escherichia coli), lower eukaryotes (such as yeast) and in vertebrates, GK is a highly conserved monomeric protein of about 200 amino acids. GK has been shown [2,3,4] to be structurally similar to the following proteins: - Protein A57R (or SalG2R) from various strains of Vaccinia virus. This protein is highly similar to GK, but contains a frameshift mutation in the N-terminal section and could therefore be inactive in that virus. The following proteins are characterized by the presence in their sequence of one or more copies of the DHR domain, a SH3 domain (see <PDOC50002> as well as a C-terminal GK-like domain, these protein are collectively termed MAGUKs (membrane-associated guanylate kinase homologs) [5]: - Drosophila lethal(1)discs large-1 tumor suppressor protein (gene dlg1). This protein is associated with septate junctions in developing flies and defects in the dlg1 gene cause neoplastic overgrowth of the imaginal disks. - Mammalian tight junction protein Zo-1. - A family of mammalian synaptic proteins that seem to interact with the cytoplasmic tail of NMDA receptor subunits. This family currently consist of SAP90/PSD-95, CHAPSYN-110/PSD-93, SAP97/DLG1 and SAP102. - Vertebrate 55 Kd erythrocyte membrane protein (p55). p55 is a palmitoylated, membrane-associated protein of unknown function. - Caenorhabditis elegans protein lin-2, which may play a structural role in the induction of the vulva. - Rat protein CASK. - Human protein DLG2. - Human protein DLG3.There is an ATP-binding site (P-loop) in the N-terminal section of GK. This region is not conserved in the GK-like domain of the above proteins which are therefore unlikely to be kinases. However these proteins retain the residues known, in GK, to be involved in the binding of GMP. As a signature pattern a highly conserved region was selected that contains two arginine and a tyrosine which are involved in GMP-binding

Consensus pattern: T-[ST]-R-x(2)-[KR]-x(2)-[DE]-x(2)-G-x(2)-Y-x-[FY]-[LIVMK]-

[1] Stehle T., Schulz G.E. J. Mol. Biol. 224:1127-1141(1992).
[2] Bryant P.J., Woods D.F. Cell 68:621-622(1992).
[3] Goebl M.G. Trends Biochem. Sci. 17:99-99(1992).
[4] Zschocke P.D., Schiltz E., Schulz G.E. Eur. J. Biochem. 213:263-269(1993).
[5] Woods D.F., Bryant P.J. Mech. Dev. 44:85-89(1994).

249. (Glyco_hydro_35)

Glycosyl hydrolases family 35 putative active site

Beta-galactosidases (EC 3.2.1.23) from mammals, fungi, plants and the bacteria Xanthomonas manihotis are evolutionary related [1,2]. They belong to family 35 in the classification of glycosyl hydrolases [3,E1].

Mammalian beta-galactosidase is a lysosomal enzyme (gene GLB1) which cleaves the terminal galactose from gangliosides, glycoproteins, and glycosaminoglycans and whose deficiency is the cause of the genetic disease Gm(1) gangliosidosis (Morquio disease type B).

On of the best conserved regions in these enzymes contains a glutamic acid residue which, on the basis of similarities with other families of glycosyl hydrolases [4], probably acts as the proton donor in the catalytic mechanism. This region wss used as a signature pattern.

Consensus pattern: G-G-P-[LIVM](2)-x(2)-Q-x-E-N-E-[FY] [The second E is the putative active site residue] Sequences known to belong to this class detected by the pattern ALL.

[1] Taron C.H., Benner J.S., Hornstra L.J., Guthrie E.P. Glycobiology 5:603-610(1995).

[2] Carey A.T., Holt K., Picard S., Wilde R., Tucker G.A., Bird C.R., Schuch W., Seymour G.B. Plant Physiol. 108:1099-1107(1995).

[3] Henrissat B., Bairoch A. Biochem. J. 293:781-788(1993).

[4] Henrissat B., Callebaut I., Fabrega S., Lehn P., Mornon J.-P., Davies G. Proc. Natl. Acad. Sci. U.S.A. 92:7090-7094(1995).

250. (Glyco_hydro_16)

Glycosyl hydrolases family 16 signature

It has been shown [1] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities:

- Bacterial beta-1,3-1,4-glucanases, or lichenases, (EC 3.2.1.73) mainly from Bacillus but also from Clostridium thermocellum (gene licB), Fibrobacter succinogenes and Rhodothermus marinus (gene bglA).
- Bacillus circulans beta-1,3-glucanase A1 (EC 3.2.1.39) (gene glcA).
- Lamarinase (EC 3.2.1.6) from Clostridium thermocellum (gene lam1).
- Streptomyces coelicolor agarase (EC 3.2.1.81) (gene dagA).
- Alteromonas carrageenovora kappa-carrageenase (EC 3.2.1.83) (gene cgkA).

Two closely clustered conserved glutamates have been shown [2] to be involved in the catalytic activity of Bacillus licheniformis lichenase. The region that contains these residues as a signature pattern was used.

Consensus pattern E-[LIV]-D-[LIV]-x(0,1)-E-x(2)-[GQ]-[KRNF]-x-[PSTA] [The two E's are active site residues]

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Juncosa M., Pons J., Dot T., Querol E., Planas A. J. Biol. Chem. 269:14530-14535(1994).

251. (Glyco_hydro_17)
Glycosyl hydrolases family 17 signature
(aka glycosyl_hydro4)

It has been shown [1,2] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities:

- Glucan endo-1,3-beta-glucosidases (EC 3.2.1.39) (endo-(1->3)-beta-glucanase) from various plants. This enzyme may be involved in the defense of plants against pathogens through its ability to degrade fungal cell wall polysaccharides.

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- Glucan 1,3-beta-glucosidase (EC 3.2.1.58) (exo-(1->3)-beta-glucanase) from yeast (gene BGL2). This enzyme may play a role in cell expansion during growth, in cell-cell fusion during mating, and in spore release during sporulation.
- Lichenases (EC 3.2.1.73) (endo-(1->3,1->4)-beta-glucanase) from various plants.

The best conserved region in the sequence of these enzymes is located in their central section. This region contains a conserved tryptophan residue which could be involved in the interaction with the glucan substrates [2] and it also contains a conserved glutamate which has been shown [3] to act as the nucleophile in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern [LIVM]-x-[LIVMFYWA](3)-[STAG]-E-[STA]-G-W-P-[STN]-x-[SAGQ] [E is an active site residue] Sequences known to belong to this class detected by the pattern ALL.

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Ori N., Sessa G., Lotan T., Himmelhoch S., Fluhr R. EMBO J. 9:3429-3436(1990).
[3] Varghese J.N., Garrett T.P.J., Colman P.M., Chen L., Hoj P.J., Fincher G.B. Proc. Natl. Acad. Sci. U.S.A. 91:2785-2789(1994).

252. (Glyco_hydro_3)
Glycosyl hydrolases family 3 active site

It has been shown [1,2] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

- Beta glucosidases (EC 3.2.1.21) from the fungi Aspergillus wentii (A-3), Hansenula anomala, Kluyveromyces fragilis, Saccharomycopsis fibuligera, (BGL1 and BGL2), Schizophyllum commune and Trichoderma reesei (BGL1).
- Beta glucosidases from the bacteria Agrobacterium tumefaciens (Cbg1), Butyrivibrio fibrisolvens (bglA), Clostridium thermocellum (bglB), Escherichia coli (bglX), Erwinia chrysanthemi (bgxA) and Ruminococcus

albus.

- Alteromonas strain O-7 beta-hexosaminidase A (EC 3.2.1.52).
- Bacillus subtilis hypothetical protein yzbA.
- Escherichica coli hypothetical protein ycfO and HI0959, the corresponding Haemophilus influenzae protein.

One of the conserved regions in these enzymes is centered on a conserved aspartic acid residue which has been shown [3], in Aspergillus wentii beta- glucosidase A3, to be implicated in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern[LIVM](2)-[KR]-x-[EQK]-x(4)-G-[LIVMFT]-[LIVT]-[LIVMF]- [ST]-D-x(2)-[SGADNI] [D is the active site residue] Sequences known to belong to this class detected by the patternALL.

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Castle L.A., Smith K.D., Morris R.O. J. Bacteriol. 174:1478-1486(1992).
[3] Bause E., Legler G. Biochim. Biophys. Acta 626:459-465(1980).

253. (Glyco_hydro_28)
Polygalacturonase active site (aka PG)

Polygalacturonase (EC 3.2.1.15) (PG) (pectinase) [1,2] catalyzes the random hydrolysis of 1,4-alpha-D-galactosiduronic linkages in pectate and other galacturonans. In fruit, polygalacturonase plays an important role in cell wall metabolism during ripening. In plant bacterial pathogens such as Erwinia carotovora or Pseudomonas solanacearum and fungal pathogens such as Aspergillus niger, polygalacturonase is involved in maceration and soft-rotting of plant tissue.

Exo-poly-alpha-D-galacturonosidase (EC 3.2.1.82) (exoPG) [3] hydrolyzes peptic acid from the non-reducing end, releasing digalacturonate.

Prokaryotic, eukaryotic PG and exoPG share a few regions of sequence similarity. The best conserved of these regions was selected. It is centered on a conserved histidine most probably involved in the catalytic mechanism [4].

Consensus pattern[GSDENKRH]-x(2)-[VMFC]-x(2)-[GS]-H-G-[LIVMAG]-x(1,2)- [LIVM]-G-S [H is the putative active site residue] Sequences known to belong to this class detected by the patternALL.

Note: these proteins belong to family 28 in the classification of glycosyl hydrolases [5].

[1] Ruttowski E., Labitzke R., Khanh N.Q., Loeffler F., Gottschalk M., Jany K.-D. Biochim. Biophys. Acta 1087:104-106(1990).
[2] Huang J., Schell M.A. J. Bacteriol. 172:3879-3887(1990).
[3] He S.Y., Collmer A. J. Bacteriol. 172:4988-4995(1990).
[4] Bussink H.J.D., Buxton F.P., Visser J. Curr. Genet. 19:467-474(1991).
[5] Henrissat B. Biochem. J. 280:309-316(1991).

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254. (Glyco_hydro_32)
Glycosyl hydrolases family 32 active site

It has been shown [1,2] that the following glycosyl hydrolases can be classified into a single family on the basis of sequence similarities:

- Inulinase (EC 3.2.1.7) (or inulase) from the fungi Kluyveromyces marxianus.
- Beta-fructofuranosidase (EC 3.2.1.26), commonly known as invertase in fungi and plants and as sucrase in bacteria (gene sacA or scrB).
- Raffinose invertase (EC 3.2.1.26) (gene rafD) from Escherichia coli plasmid pRSD2.
- Levanase (EC 3.2.1.65) (gene sacC) from Bacillus subtilis.

One of the conserved regions in these enzymes is located in the N-terminal section and contains an aspartic acid residue which has been shown [3], in yeast invertase to be important for the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern H-x(2)-P-x(4)-[LIVM]-N-D-P-N-G [D is the active site residue]
Sequences known to belong to this class detected by the patternALL.

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Gunasekaran P., Karunakaran T., Cami B., Mukundan A.G., Preziosi L., Baratti J. J. Bacteriol. 172:6727-6735(1990).
[3] Reddy V.A., Maley F. J. Biol. Chem. 265:10817-10120(1990).

255. (Glyco_hydro_1)
Glycosyl hydrolases family 1 signatures

It has been shown [1 to 4] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

- Beta-glucosidases (EC 3.2.1.21) from various bacteria such as Agrobacterium strain ATCC 21400, Bacillus polymyxa, and Caldocellum saccharolyticum.
- Two plants (clover) beta-glucosidases (EC 3.2.1.21).
- Two different beta-galactosidases (EC 3.2.1.23) from the archaebacteria Sulfolobus solfataricus (genes bgaS and lacS).
- 6-phospho-beta-galactosidases (EC 3.2.1.85) from various bacteria such as Lactobacillus casei, Lactococcus lactis, and Staphylococcus aureus.
- 6-phospho-beta-glucosidases (EC 3.2.1.86) from Escherichia coli (genes bglB and ascB) and from Erwinia chrysanthemi (gene arbB).
- Plants myrosinases (EC 3.2.3.1) (sinigrinase) (thioglucosidase).
- Mammalian lactase-phlorizin hydrolase (LPH) (EC 3.2.1.108 / EC 3.2.1.62). LPH, an integral membrane glycoprotein, is the enzyme that splits lactose in the small intestine. LPH is a large protein of about 1900 residues which contains four tandem repeats of a domain of about 450 residues which is

evolutionary related to the above glycosyl hydrolases.

One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [5], in the beta-glucosidase from Agrobacterium, to be directly involved in glycosidic bond cleavage by acting as a nucleophile. This region was used as a signature pattern. As a second signature pattern we selected a conserved region, found in the N-terminal extremity of these enzymes, this region also contains a glutamic acid residue.

Consensus pattern[LIVMFSTC]-[LIVFYS]-[LIV]-[LIVMST]-E-N-G-[LIVMFAR]-[CSAGN] [E is the active site residue] Sequences known to belong to this class detected by the patternALL.

Note: this pattern will pick up the last two domains of LPH; the first two domains, which are removed from the LPH precursor by proteolytic processing, have lost the active site glutamate and may therefore be inactive [4].

Consensus patternF-x-[FYWM]-[GSTA]-x-[GSTA]-x-[GSTA](2)-[FYNH]-[NQ]-x-E-x-[GSTA] Sequences known to belong to this class detected by the pattern ALL.

Note: this pattern will pick up the last three domains of LPH.

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Henrissat B. Protein Seq. Data Anal. 4:61-62(1991).
[3] Gonzalez-Candelas L., Ramon D., Polaina J. Gene 95:31-38(1990).
[4] El Hassouni M., Henrissat B., Chippaux M., Barras F. J. Bacteriol. 174:765-777(1992).
[5] Withers S.G., Warren R.A.J., Street I.P., Rupitz K., Kempton J.B., Aebersold R. J. Am. Chem. Soc. 112:5887-5889(1990).

256. Glyco_hydro_20
Glycosyl hydrolase family 20
Previous Pfam IDs: glycosyl_hydr11;
Number of members: 33

257. (Glyco_hydro_9)
Glycosyl hydrolases family 9 active sites signatures
(aka Glycosyl_hydr12)

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family E [3] or as the glycosyl hydrolases family 9 [4,E1]. The enzymes which are currently known to belong to this family are listed below.

- Butyrivibrio fibrisolvens cellodextrinase 1 (ced1).
- Cellulomonas fimi endoglucanases B (cenB) and C (cenC).
- Clostridium cellulolyticum endoglucanase G (celCCG).
- Clostridium cellulovorans endoglucanase C (engC).
- Clostridium stercoararium endoglucanase Z (avicelase I) (celZ).
- Clostridium thermocellum endoglucanases D (celD), F (celF) and I (celI).
- Fibrobacter succinogenes endoglucanase A (endA).
- Pseudomonas fluorescens endoglucanase A (celA).
- Streptomyces reticuli endoglucanase 1 (cel1).
- Thermomonospora fusca endoglucanase E-4 (celD).

- Dictyostelium discoideum spore germination specific endoglucanase 270-6. This slime mold enzyme may digest the spore cell wall during germination, to release the enclosed amoeba.
- Endoglucanases from plants such as Avocado or French bean. In plants this enzyme may be involved the fruit ripening process.

Two of the most conserved regions in these enzymes are centered on conserved residues which have been shown [5,6], in the endoglucanase D from Cellulomonas thermocellum, to be important for the catalytic activity. The first region contains an active site histidine and the second region contains two catalytically important residues: an aspartate and a glutamate. Both regions were used as signature patterns.

Consensus pattern [STV]-x-[LIVMFY]-[STV]-x(2)-G-x-[NKR]-x(4)-[PLIVM]-H-x-R [H is an active site residue] Sequences known to belong to this class detected by the pattern ALL, except for Cellulomonas fimi cenC and Streptomyces reticuli cel1.

Consensus pattern [FYW]-x-D-x(4)-[FYW]-x(3)-E-x-[STA]-x(3)-N-[STA] [D and E are active site residues] Sequences known to belong to this class detected by the pattern ALL, except for Fibrobacter succinogenes endA whose sequence seems to be incorrect.

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).
[2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).
[3] Henrissat B., Claeyssens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).
[4] Henrissat B. Biochem. J. 280:309-316(1991).
[5] Tomme P., Chauvaux S., Beguin P., Millet J., Aubert J.-P., Claeyssens M. J. Biol. Chem. 266:10313-10318(1991).
[6] Tomme P., van Beeumen J., Claeyssens M. Biochem. J. 285:319-324(1992).

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258. Matrix protein (MA), p15 (GAG_ma)

The matrix protein, p15, is encoded by the gag gene. MA is involved in pathogenicity [1].

[1] : Pozsgay JM, Beilharz MW, Wines BD, Hess AD, Pitha PM, J Virol 1993;67:5989-5999.

259. Gag polyprotein, inner coat protein p12 (GAG_P12)

The retroviral p12 is a virion structural protein. p12 is proline rich. The function carried out by p12 in assembly and replication is unknown. p12C is associated with pathogenicity of the virus

[1] Pozsgay JM, Beilharz MW, Wines BD, Hess AD, Pitha PM, J Virol 1993;67:5989-5999.

260. Glutamine synthetase signatures (GLN-SYNT)

Glutamine synthetase (EC <u>6.3.1.2</u>) (GS) [1] plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine. There seem to be three different classes of GS [2,3,4]: - Class I enzymes (GSI) are specific to prokaryotes, and are oligomers of 12 identical subunits. The activity of GSI-type enzyme is controlled by the adenylation of a tyrosine residue. The adenylated enzyme is inactive. - Class II enzymes (GSII) are found in eukaryotes and in bacteria belonging to the Rhizobiaceae, Frankiaceae, and Streptomycetaceae families (these bacteria have also a class-I GS). GSII are octamer of identical subunits. Plants have two or more isozymes of GSII, one of the isozymes is translocated into the chloroplast. - Class III enzymes (GSIII) has, currently, only been found in Bacteroides fragilis and in butyrivibrio fibrisolvens. It is a hexamer of identical chains. It is much larger (about 700 amino acids) than the GSI (450 to 470 amino acids) or GSII (350 to 420 amino acids) enzymes. While the three classes of GS's are clearly structurally related, the sequence similarities are not so extensive. As signature patterns three conserved regions were selected. The first pattern is based on a conserved tetrapeptide in the N-terminal section of the enzyme, the second one is based on a glycine-rich region which is thought to be involved in ATP-binding. The third pattern is specific to class I glutamine synthetases and includes the tyrosine residue which is reversibly adenylated.

Consensus pattern: [FYWL]-D-G-S-S-x(6,8)-[DENQSTAK]-[SA]-[DE]-x(2)-[LIVMFY]-
Consensus pattern: K-P-[LIVMFYA]-x(3,5)-[NPAT]-G-[GSTAN]-G-x-H-x(3)-S-
Consensus pattern: K-[LIVM]-x(5)-[LIVMA]-D-[RK]-[DN]-[LI]-Y [Y is the site of adenylation]-

[1] Eisenberg D., Almassy R.J., Janson C.A., Chapman M.S., Suh S.W., Cascio D., Smith W.W. Cold Spring Harbor Symp. Quant. Biol. 52:483-490(1987).
[2] Kumada Y., Benson D.R., Hillemann D., Hosted T.J., Rochefort D.A., Thompson C.J., Wohlleben W., Tateno Y. Proc. Natl. Acad. Sci. U.S.A. 90:3009-3013(1993).
[3] Shatters R.G., Kahn M.L. J. Mol. Evol. 29:422-428(1989).

[4] Brown J.R., Masuchi Y., Robb F.T., Doolittle W.F. J. Mol. Evol. 38:566-576(1994).

261. Globins profile (globin1)

Globins are heme-containing proteins involved in binding and/or transporting oxygen [1]. They belong to a very large and well studied family which is widely distributed in many organisms. The major groups of globins are: - Hemoglobins (Hb) from vertebrates. Hb is the protein responsible for transporting oxygen from the lungs to other tissues. It is a tetramer of two alpha and two beta chains. Most vertebrate species also express specific embryonic or fetal forms of hemoglobin where the alpha or the beta chains are replaced by a chain with higher oxygen affinity, as for the gamma, delta, epsilon and zeta chains in mammals, for example. - Myoglobins (Mg) from vertebrates. Mg is a monomeric protein responsible for oxygen storage in muscles. - Invertebrate globins [2]. A wide variety of globins are found in invertebrates. Molluscs generally have one or two muscle globins which are either monomeric or dimeric. Insects, such as the midge Chironomus thummi, have a large set of extracellular globins. Nematodes and annelids have a variety of intracellular and extracellular globins; some of them are multi- domain polypeptides (from two up to nine-domain globins) and some produce large, disulfide-bonded aggregates. - Leghemoglobins (Lg) from the root nodules of leguminous plants. Lg provides oxygen for bacteroids. - Flavohemoproteins from bacteria (Escherichia coli hmpA) and fungi [3]. These proteins consist of two distinct domains: an N-terminal globin domain and a C-terminal FAD-containing reductase domain. In bacteria such as Vitreoscilla, the enzyme-associated globin is a single domain protein. All these globins seem to have evolved from a common ancestor. The profile developed to detect members of the globin family is based on a structural alignment of selected globin sequences [1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).[2] Goodman M., Pedwaydon J., Czelusniak J., Suzuki T., Gotoh T., Moens L., Shishikura F., Walz D., Vinogradov S. J. Mol. Evol. 27:236-249(1988).

Plant hemoglobins signature (globin2)

Leghemoglobins [1] are hemoproteins present in the root nodules of leguminousplants. Leghemoglobins are structurally and functionally related to hemoglobin and myoglobin. By providing oxygen to the bacteroids, they are essential for symbiotic nitrogen fixation. Structurally related hemoglobins from the nodules of non-leguminous plants [2,3], and from the roots of non-nodulating plants[4] have been recently sequenced. A signature pattern was developed that picks up the sequence of plants hemoglobins, exclusively.

Consensus pattern: [SN]-P-x-L-x(2)-H-A-x(3)-F-

[1] Powell R., Gannon F. BioEssays 9:117-121(1988).
[2] Kortt A.A., Trinick M.J., Appleby C.A. Eur. J. Biochem. 175:141-149(1988).
[3] Kortt A.A., Inglis A.S., Fleming A.I., Appleby C.A. FEBS Lett. 231:341-346(1988).
[4] Bogusz D., Appleby C.A., Landsmann J., Dennis E.S., Trinick M.J., Peacock W.J. Nature 331:178-180(1988).

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262. Fructose-bisphosphate aldolase class-I active site (glycolytic_enz)

Fructose-bisphosphate aldolase [1,2] is a glycolytic enzyme that catalyzes the reversible aldol cleavage or condensation of fructose-1,6-bisphosphate into dihydroxyacetone-phosphate and glyceraldehyde 3-phosphate.There are two classes of fructose-bisphosphate aldolases with different catalytic mechanisms. Class-I aldolases [3], mainly found in higher eukaryotes, are homotetrameric enzymes which form a Schiff-base intermediate between the C-2 carbonyl group of the substrate (dihydroxyacetone phosphate)and the epsilon-amino group of a lysine residue. In vertebrates, three forms of this enzyme are found: aldolase A in muscle, aldolase B in liver and aldolase C in brain. The sequence around the lysine involved in the Schiff-base is highly conserved and can be used as a signature for this class of enzyme.

Consensus pattern: [LIVM]-x-[LIVMFYW]-E-G-x-[LS]-L-K-P-[SN] [K is involved in Schiff-base formation]-

[1] Perham R.N. Biochem. Soc. Trans. 18:185-187(1990).
[2] Marsh J.J., Lebherz H.G. Trends Biochem. Sci. 17:110-113(1992).
[3] Freemont P.S., Dunbar B., Fothergill-Gilmore L.A. Biochem. J. 249:779-788(1988).

263. Glycosyl hydrolases family 11 active sites signatures

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family G [3] or as the glycosyl hydrolases family 11 [4,E1]. The enzymes which are currently known to belong to this family are listed below. - Aspergillus awamori xylanase C (xynC). - Bacillus circulans, pumilus, stearothermophilus and subtilis xylanase (xynA). - Clostridium acetobutylicum xylanase (xynB). - Clostridium stercorarium xylanase A (xynA). - Fibrobacter succinogenes xylanase C (xynC) which consist of two catalytic domains that both belong to family 10. - Neocallimastix patriciarum xylanase A (xynA). - Ruminococcus flavefaciens bifunctional xylanase XYLA (xynA). This protein consists of three domains: a N-terminal xylanase catalytic domain that belongs to family 11 of glycosyl hydrolases; a central domain composed of short repeats of Gln, Asn an Trp, and a C-terminal xylanase catalytic domain that belongs to family 10 of glycosyl hydrolases. - Schizophyllum commune xylanase A. - Streptomyces lividans xylanases B (xlnB) and C (xlnC). - Trichoderma reesei xylanases I and II. Two of the conserved regions in these enzymes are centered on glutamic acidresidues which have both been shown [5], in Bacillus pumilis xylanase, to be necessary for catalytic activity. Both regions were used as signature patterns.

Consensus pattern: [PSA]-[LQ]-x-E-Y-Y-[LIVM](2)-[DE]-x-[FYWHN] [E is an active site residue]-

Consensus pattern: [LIVMF]-x(2)-E-[AG]-[YWG]-[QRFGS]-[SG]-[STAN]-G-x-[SAF] [E is an active site residue]-

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).

[2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[3] Henrissat B., Claeyssens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).

[4] Henrissat B. Biochem. J. 280:309-316(1991).

[5] Ko E.P., Akatsuka H., Moriyama H., Shinmyo A., Hata Y., Katsube Y., Urabe I., Okada H. Biochem. J. 288:117-121(1992).

264. Glycosyl hydrolase family 14

This family are beta amylases.

265. Glycosyl hydrolases family 1 signatures

It has been shown [1 to 4] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family: - Beta-glucosidases (EC 3.2.1.21) from various bacteria such as Agrobacterium strain ATCC 21400, Bacillus polymyxa, and Caldocellum saccharolyticum. - Two plants (clover) beta-glucosidases (EC 3.2.1.21). - Two different beta-galactosidases (EC 3.2.1.23) from the archaebacteria Sulfolobus solfataricus (genes bgaS and lacS). - 6-phospho-beta-galactosidases (EC 3.2.1.85) from various bacteria such as Lactobacillus casei, Lactococcus lactis, and Staphylococcus aureus. - 6-phospho-beta-glucosidases (EC 3.2.1.86) from Escherichia coli (genes bglB and ascB) and from Erwinia chrysanthemi (gene arbB). - Plants myrosinases (EC 3.2.3.1) (sinigrinase) (thioglucosidase). - Mammalian lactase-phlorizin hydrolase (LPH) (EC 3.2.1.108 / EC 3.2.1.62). LPH, an integral membrane glycoprotein, is the enzyme that splits lactose in the small intestine. LPH is a large protein of about 1900 residues which contains four tandem repeats of a domain of about 450 residues which is evolutionary related to the above glycosyl hydrolases. One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [5], in the beta-glucosidase from Agrobacterium, to be directly involved in glycosidic bond cleavage by acting as a nucleophile. This region was used as a signature pattern. As a second signature pattern a conserved region was selected, found in the N-terminal extremity of these enzymes, this region also contains a glutamic acid residue.

Consensus pattern: [LIVMFSTC]-[LIVFYS]-[LIV]-[LIVMST]-E-N-G-[LIVMFAR]-[CSAGN] [E is the active site residue]

Note: this pattern will pick up the last two domains of LPH; the first two domains, which are removed from the LPH precursor by proteolytic processing, have lost the active site glutamate and may therefore be inactive [4].

Consensus pattern: F-x-[FYWM]-[GSTA]-x-[GSTA]-x-[GSTA](2)-[FYNH]-[NQ]-x-E-x-[GSTA]-

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Henrissat B. Protein Seq. Data Anal. 4:61-62(1991).
[3] Gonzalez-Candelas L., Ramon D., Polaina J. Gene 95:31-38(1990).
[4] El Hassouni M., Henrissat B., Chippaux M., Barras F. J. Bacteriol. 174:765-777(1992).
[5] Withers S.G., Warren R.A.J., Street I.P., Rupitz K., Kempton J.B., Aebersold R. J. Am. Chem. Soc. 112:5887-5889(1990).

266. Glycosyl hydrolases family 2 signatures

It has been shown [1,2,E1] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family: - Beta-galactosidases (EC 3.2.1.23) from bacteria such as Escherichia coli (genes lacZ and ebgA), Clostridium acetobutylicum, Clostridium thermosulfurogenes, Klebsiella pneumoniae, Lactobacillus delbrueckii, or Streptococcus thermophilus and from the fungi Kluyveromyces lactis. - Beta-glucuronidase (EC 3.2.1.31) from Escherichia coli (gene uidA) and from mammals. One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [3], in Escherichia coli lacZ, to be the general acid/base catalyst in the active site of the enzyme. This region was used as a signature pattern. As a second signature pattern a highly conserved region was selected located some sixty residues upstream from the active site glutamate.

Consensus pattern: N-x-[LIVMFYWD]-R-[STACN](2)-H-Y-P-x(4)-[LIVMFYWS](2)-x(3)-[DN]-x(2)-G-[LIVMFYW](4)-
Consensus pattern: [DENQLF]-[KRVW]-N-[HRY]-[STAPV]-[SAC]-[LIVMFS](3)-W-[GS]-x(2,3)-N-E [E is the active site residue]-

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Schroeder C.J., Robert C., Lenzen G., McKay L.L., Mercenier A. J. Gen. Microbiol. 137:369-380(1991).
[3] Gebler J.C., Aebersold R., Withers S.G. J. Biol. Chem. 267:11126-11130(1992).

267. Glycosyl hydrolases family 3 active site

It has been shown [1,2] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

- Beta glucosidases (EC 3.2.1.21) from the fungi Aspergillus wentii (A-3), Hansenula anomala, Kluyveromyces fragilis, Saccharomycopsis fibuligera, (BGL1 and BGL2), Schizophyllum commune and Trichoderma reesei (BGL1).
- Beta glucosidases from the bacteria Agrobacterium tumefaciens (Cbgl), Butyrivibrio fibrisolvens (bglA), Clostridium thermocellum (bglB), Escherichia coli (bglX), Erwinia chrysanthemi (bgxA) and Ruminococcus albus. - Alteromonas strain O-7 beta-hexosaminidase A (EC 3.2.1.52).
- Bacillus subtilis hypothetical protein yzbA.
- Escherichica coli hypothetical protein ycfO and HI0959, the corresponding Haemophilus influenzae protein.

One of the conserved regions in these enzymes is centered on a conserved aspartic acid residue which has been shown [3], in Aspergillus wentii beta-glucosidase A3, to be implicated in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern: [LIVM](2)-[KR]-x-[EQK]-x(4)-G-[LIVMFT]-[LIVT]-[LIVMF]-[ST]-D-x(2)-[SGADNI] [D is the active site residue]

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Castle L.A., Smith K.D., Morris R.O. J. Bacteriol. 174:1478-1486(1992).
[3] Bause E., Legler G. Biochim. Biophys. Acta 626:459-465(1980).

268. Glycosyl hydrolases family 8 signature

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91)(exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family D [3] or as the glycosyl hydrolases family 8 [4,E1]. The enzymes which are currently known to belong to this family are listed below. - Acetobacter xylinum endonuclease cmcAX. - Bacillus strain KSM-330 acidic endonuclease K (Endo-K). - Cellulomonas josui endoglucanase 2 (celB). - Cellulomonas uda endoglucanase. - Clostridium cellulolyticum endoglucanases C (celcCC). - Clostridium thermocellum endoglucanases A (celA). - Erwinia chrysanthemi minor endoglucanase y (celY). - Bacillus circulans beta-glucanase (EC 3.2.1.73). - Escherichia coli hypothetical protein yhjM. The most conserved region in these enzymes is a stretch of about 20 residues that contains two conserved aspartate. The first asparatate is thought [5] to act as the nucleophile in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern: A-[ST]-D-[AG]-D-x(2)-[IM]-A-x-[SA]-[LIVM]-[LIVMG]-x-A- x(3)-[FW] [The first D is an active site residue]-

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).
[2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).
[3] Henrissat B., Claeyssens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).
[4] Henrissat B. Biochem. J. 280:309-316(1991).
[5] Alzari P.M., Souchon H., Dominguez R. Structure 4:265-275(1996).

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269. Glycosyl hydrolases family 9 active sites signatures

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produce a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family E [3] or as the glycosyl hydrolases family 9 [4,E1]. The enzymes which are currently known to belong to this family are listed below. - Butyrivibrio fibrisolvens cellodextrinase 1 (ced1). - Cellulomonas fimi endoglucanases B (cenB) and C (cenC). - Clostridium cellulolyticum endoglucanase G (celCCG). - Clostridium cellulovorans endoglucanase C (engC). - Clostridium stercoararium endoglucanase Z (avicelase I) (celZ). - Clostridium thermocellum endoglucanases D (celD), F (celF) and I (celI). - Fibrobacter succinogenes endoglucanase A (endA). - Pseudomonas fluorescens endoglucanase A (celA). - Streptomyces reticuli endoglucanase 1 (cel1). -

Thermomonospora fusca endoglucanase E-4 (celD). - Dictyostelium discoideum spore germination specific endoglucanase 270-6. This slime mold enzyme may digest the spore cell wall during germination, to release the enclosed amoeba. - Endoglucanases from plants such as Avocado or French bean. In plants this enzyme may be involved the fruit ripening process. Two of the most conserved regions in these enzymes are centered on conserved residues which have been shown [5,6], in the endoglucanase D from Cellulomonas thermocellum, to be important for the catalytic activity. The first region contains an active site histidine and the second region contains two catalytically important residues: an aspartate and a glutamate. Both regions were used as signature patterns.

Consensus pattern: [STV]-x-[LIVMFY]-[STV]-x(2)-G-x-[NKR]-x(4)-[PLIVM]-H-x-R [H is an active site residue]-

Consensus pattern: [FYW]-x-D-x(4)-[FYW]-x(3)-E-x-[STA]-x(3)-N-[STA] [D and E are active site residues]-

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).

[2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[3] Henrissat B., Claeyssens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).

[4] Henrissat B. Biochem. J. 280:309-316(1991).

[5] Tomme P., Chauvaux S., Beguin P., Millet J., Aubert J.-P., Claeyssens M. J. Biol. Chem. 266:10313-10318(1991).

[6] Tomme P., van Beeumen J., Claeyssens M. Biochem. J. 285:319-324(1992).

270. Glyceraldehyde 3-phosphate dehydrogenase active site (gpdh)

Glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH) [1] is a tetrameric NAD-binding enzyme common to both the glycolytic and gluconeogenic pathways. A cysteine in the middle of the molecule is involved in forming a covalent phosphoglycerol thioester intermediate. The sequence around this cysteine is totally conserved in eubacterial and eukaryotic GAPDHs and is also present, albeit in a variant form, in the otherwise highly divergent archaebacterial GAPDH [2].Escherichia coli D-erythrose 4-phosphate dehydrogenase (E4PDH) (gene epd orgapB) is an enzyme highly related to GAPDH [3].

Consensus pattern: [ASV]-S-C-[NT]-T-x(2)-[LIM] [C is the active site residue]-

[1] Harris J.I., Waters M. (In) The Enzymes (3rd edition) 13:1-50(1976).
[2] Fabry S., Lang J., Niermann T., Vingron M., Hensel R. Eur. J. Biochem. 179:405-413(1989).
[3] Zhao G., Pease A.J., Bharani N., Winkler M.E. J. Bacteriol. 177:2804-2812(1995).

271. Granulins signature

Granulins [1] are a family of cysteine-rich peptides of about 6 Kd which may have multiple biological activity. A precursor protein (known as acrogranin) potentially encodes seven different forms of granulin (grnA to grnG) which are probably released by post-translational proteolytic processing. A schematic representation of the structure of a granulin is shown below: xxxCxxxxxCxxxxxCCxxxxxxxxCCxxxxxxCCxxxxxCCxxxxxCxxxxxxCx *************'C': conserved cysteine probably involved in a disulfide bond.'*': position of the pattern. Granulins are evolutionary related to a PMP-D1, a peptide extracted from thepars intercerebralis of migratory locusts [2].

Consensus pattern: C-x-D-x(2)-H-C-C-P-x(4)-C [The four C's are probably involved in disulfide bonds]-

[1] Bhandari V., Palfree R.G., Bateman A. Proc. Natl. Acad. Sci. U.S.A. 89:1715-1719(1992).
[2] Nakakura N., Hietter H., van Dorsselaer A., Luu B. Eur. J. Biochem. 204:147-153(1992).

272. (HCV RdRp) Hepatitis C virus RNA dependent RNA polymerase

The RNA dependent RNA polymerase is also known as non-structural protein NS5B. NS5B is a 65 kDa protein that resembles other viral RNA polymerases. HCV replication is thought to occur in membrane bound replication complexes. These complexes transcribe the positive strand and the resulting minus strand is used as a template for the synthesis of genomic RNA. There are two viral proteins involved in the reaction, NS3 and NS5B.[1,2]

[1] Lohmann V, Korner F, Herian U, Bartenschlager R; J Virol 1997;71:8416-8428. [2] Behrens SE, Tomei L, De Francesco R; EMBO J 1996;15:12-22. [3] Ishido S, Fujita T, Hotta H; Biochem Biophys Res Commun 1998;244:35-40.

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273. (HHH) Helix-hairpin-helix motif.

[1] Doherty AJ, Serpell LC, Ponting CP; Nucleic Acids Res 1996;24:2488-2497.

274. HIT family signature

Recently a family of small proteins of about 12 to 16 Kd has been described[1]. This family currently consists of: - Mammalian protein HINT (also known as Protein kinase C inhibitor 1 or PKCI- 1). HINT was incorrectly thought to be a specific inhibitor of PKC. It has been shown to bind zinc. - Fission yeast diadenosine 5',5'''-P1,P4-tetraphosphate asymmetrical hydrolase (Ap4Aase) (EC 3.6.1.17) [2] (gene aph1), which cleaves A-5'-PPPP- 5'A to yield AMP and ATP. - FHIT, a human protein whose gene is altered in different tumors and which acts [3] as a diadenosine 5',5'''-P1,P3-triphosphate hydrolase (Ap3Aase) (EC 3.6.1.29) cleaving A-5'-PPP-5'A to yield AMP and ADP. - Yeast proteins HNT1 and HNT2. - Maize zinc-binding protein ZBP14. - Escherichia coli hypothetical protein ycfF. - Haemophilus influenzae hypothetical protein HI0961. - Helicobacter pylori hypothetical protein HP0404. - Methanococcus jannaschii hypothetical protein MJ0866. - Mycobacterium leprae hypothetical protein U296A. - Synechocystis strain PCC 6803 hypothetical protein slr1234. - Caenorhabditis elegans hypothetical protein F21C3.3. - A hypothetical 13.2 Kd protein in hisE 3'region in Azospirillum brasilense. - A hypothetical 13.1 Kd protein in p37 5'region in Mycoplasma hyorhinis. - A hypothetical 12.4 Kd protein in psbAII 5'region in Synechococcus strain PCC 7942.All these proteins contains a region with three clustered histidines. This region is responsible for the designation of this family: HIT, for 'HIstidineTriad [1]. This region was originally thought to be implied in the binding of a zinc ion but was later identified [4] as part of the alpha-phosphate binding site of a nucleotide-binding domain. As a signature pattern, the region of the histidine triad was selected.

Consensus pattern: [NQA]-x(4)-[GAV]-x-[QF]-x-[LIVM]-x-H-[LIVMFYT]-H-[LIVMFT]-H-[LIVMF](2)-[PSGA]-

[1] Seraphin B. DNA Seq. 3:177-179(1992).
[2] Huang Y., Garrison P.N., Barnes L.D. Biochem. J. 312:925-932(1995).
[3] Barnes L.D., Garrison P.N., Siprashvili Z., Guranowski A., Robinson A.K., Ingram S.W., Croce C.M., Ohta M., Huebner K. Biochemistry 35:11529-11535(1996).
[4] Brenner C., Garrison P., Gilmour J., Peisach D., Ringe D., Petsko G.A., Lowenstein J.M. Nat. Struct. Biol. 4:231-238(1997).

275. Myc-type, 'helix-loop-helix' dimerization domain signature (HLH)

A number of eukaryotic proteins, which probably are sequence specific DNA-binding proteins that act as transcription factors, share a conserved domain of 40 to 50 amino acid residues. It has been proposed [1] that this domain is formed of two amphipathic helices joined by a variable length linker region that could form a loop. This 'helix-loop-helix' (HLH) domain mediates protein dimerization and has been found in the proteins listed below [2,3,E1,E2]. Most of these proteins have an extra basic region of about 15 amino acid residues that is adjacent to the HLH domain and specifically binds to DNA. They are refered as basic helix-loop-helix proteins (bHLH), and are classified in two groups: class A (ubiquitous) and class B (tissue-specific). Members of the bHLH family bind variations on the core sequence 'CANNTG', also referred to as the E-box motif. The homo- or heterodimerization mediated by the HLH domain is independent of, but necessary for DNA binding, as two basic regions are required for DNA binding activity. The HLH proteins lacking the basic domain (Emc, Id) function as negative regulators since they form heterodimers, but fail to bind DNA. The hairy-related proteins (hairy, E(spl), deadpan) also repress transcription although they can bind DNA. The proteins of this subfamily act together with co-repressor proteins, like groucho, through their C-terminal motif WRPW. - The myc family of cellular oncogenes [4], which is currently known to contain four members: c-myc [E3], N-myc, L-myc, and B-myc. The myc genes are thought to play a role in cellular differentiation and proliferation. - Proteins involved in myogenesis (the induction of muscle cells). In mammals MyoD1 (Myf-3), myogenin (Myf-4), Myf-5, and Myf-6 (Mrf4 or herculin), in birds CMD1 (QMF-1), in Xenopus MyoD and MF25, in Caenorhabditis elegans CeMyoD, and in Drosophila nautilus (nau). - Vertebrate proteins that bind specific DNA sequences ('E boxes') in various immunoglobulin chains enhancers: E2A or ITF-1 (E12/pan-2 and E47/pan-1), ITF-2 (tcf4), TFE3, and TFEB. - Vertebrate neurogenic differentiation factor 1 that acts as differentiation factor during neurogenesis. - Vertebrate MAX protein, a transcription regulator that forms a sequence- specific DNA-binding protein complex with myc or mad. - Vertebrate Max Interacting Protein 1 (MXI1 protein) which acts as a transcriptional repressor and may antagonize myc transcriptional activity by competing for max. - Proteins of the bHLH/PAS superfamily which are transcriptional activators. In mammals, AH receptor nuclear translocator (ARNT), single-minded homologs (SIM1 and SIM2), hypoxia-inducible factor 1 alpha (HIF1A), AH receptor (AHR), neuronal pas domain proteins (NPAS1 and NPAS2), endothelial pas domain protein 1 (EPAS1), mouse ARNT2, and human BMAL1. In drosophila, single-minded (SIM), AH receptor nuclear translocator (ARNT), trachealess protein (TRH), and similar protein (SIMA). - Mammalian transcription factors HES, which repress transcription by acting on two types of DNA sequences, the E box and the N box. - Mammalian MAD protein (max dimerizer) which acts as transcriptional repressor and may antagonize myc transcriptional activity by competing for max. - Mammalian Upstream Stimulatory Factor 1 and 2 (USF1 and USF2), which bind to a symmetrical DNA sequence that is found in a variety of viral and cellular promoters. - Human lyl-1 protein; which is involved, by chromosomal translocation, in T- cell leukemia. - Human transcription factor AP-4. - Mouse helix-loop-helix proteins MATH-1 and MATH-2 which activate E box- dependent transcription in collaboration with E47. - Mammalian stem cell protein (SCL) (also known as tal1), a protein which may play an important role in hemopoietic differentiation. SCL is involved, by chromosomal translocation, in stem-cell leukemia. - Mammalian proteins Id1 to Id4 [5]. Id (inhibitor of DNA binding) proteins lack a basic DNA-binding domain but are able to form heterodimers with other HLH proteins, thereby inhibiting binding to DNA. - Drosophila extra-macrochaetae (emc) protein, which participates in sensory organ patterning by antagonizing the neurogenic activity of the achaete- scute complex. Emc is the homolog of mammalian Id proteins. - Human Sterol

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Regulatory Element Binding Protein 1 (SREBP-1), a transcriptional activator that binds to the sterol regulatory element 1 (SRE-1) found in the flanking region of the LDLR gene and in other genes. - Drosophila achaete-scute (AS-C) complex proteins T3 (l'sc), T4 (scute), T5 (achaete) and T8 (asense). The AS-C proteins are involved in the determination of the neuronal precursors in the peripheral nervous system and the central nervous system. - Mammalian homologs of achaete-scute proteins, the MASH-1 and MASH-2 proteins. - Drosophila atonal protein (ato) which is involved in neurogenesis. - Drosophila daughterless (da) protein, which is essential for neurogenesis and sex-determination. - Drosophila deadpan (dpn), a hairy-like protein involved in the functional differentiation of neurons. - Drosophila delilah (dei) protein, which is plays an important role in the differentiation of epidermal cells into muscle. - Drosophila hairy (h) protein, a transcriptional repressor which regulates the embryonic segmentation and adult bristle patterning. - Drosophila enhancer of split proteins E(spl), that are hairy-like proteins active during neurogenesis. also act as transcriptional repressors. - Drosophila twist (twi) protein, which is involved in the establishment of germ layers in embryos. - Maize anthocyanin regulatory proteins R-S and LC. - Yeast centromere-binding protein 1 (CPF1 or CBF1). This protein is involved in chromosomal segregation. It binds to a highly conserved DNA sequence, found in centromers and in several promoters. - Yeast INO2 and INO4 proteins. - Yeast phosphate system positive regulatory protein PHO4 which interacts with the upstream activating sequence of several acid phosphatase genes. - Yeast serine-rich protein TYE7 that is required for ty-mediated ADH2 expression. - Neurospora crassa nuc-1, a protein that activates the transcription of structural genes for phosphorus acquisition. - Fission yeast protein esc1 which is involved in the sexual differentiation process. The schematic representation of the helix-loop-helix domain is shown here: xxxxxxxxxxxxxxxxxxxxxxxxx-------------------xxxxxxxxxxxxxxxxxxxxxxxx Amphipathic helix 1 Loop Amphipathic helix 2. The signature pattern developed to detect this domain spans completely the second amphipathic helix.

Consensus pattern: [DENSTAP]-[KTR]-[LIVMAGSNT]-{FYWCPHKR}-[LIVMT]-[LIVM]- x(2)-[STAV]-[LIVMSTACKR]-x-[VMFYH]-[LIVMTA]-{P}-{P}-[LIVMRKHQ].-

[1] Murre C., McCaw P.S., Baltimore D. Cell 56:777-783(1989).

[2] Garrel J., Campuzano S. BioEssays 13:493-498(1991).

[3] Kato G.J., Dang C.V. FASEB J. 6:3065-3072(1992).

[4] Krause M., Fire A., Harrison S.W., Priess J., Weintraub H. Cell 63:907-919(1990).

[5] Riechmann V., van Cruechten I., Sablitzky F. Nucleic Acids Res. 22:749-755(1994).

276. HMG14 and HMG17 signature

High mobility group (HMG) proteins are a family of relatively low molecular weight non-histone components in chromatin. HMG14 and HMG17 [1], two related proteins of about 100 amino acid residues, bind to the inner side of the nucleosomal DNA thus altering the interaction between the DNA and the histone octamer. These two proteins may be involved in the process which maintains transcribable genes in a unique chromatin conformation. The trout nonhistone chromosomal protein H6 (histone T) also belongs to this family. As a signature pattern a conserved stretch of 10 residues located in the N-terminal section of HMG14 and HMG17 was selected.

Consensus pattern: R-R-S-A-R-L-S-A-[RK]-P-

[1] Bustin M., Reeves R. Prog. Nucleic Acid Res. Mol. Biol. 54:35-100(1996).

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277. Hydroxymethylglutaryl-coenzyme A lyase active site (HMGL1)

3-hydroxy-3-methylglutaryl-coenzyme A lyase (HMG-CoA lyase or HL) (EC 4.1.3.4)catalyzes the transformation of HMG-CoA into acetyl-CoA and acetoacetate. In vertebrates it is a mitochondrial enyme which is involved in ketogenesis and in leucine catabolism [1]. In some bacteria, such as Pseudomonas mevalonii, it is involved in mevalonate catabolism (gene mvaB). A cysteine has been shown[2], in mvaB, to be required for the activity of the enzyme. The region around this residue is perfectly conserved and is used as a signature pattern.

Consensus pattern: S-V-A-G-L-G-G-C-P-Y [C is the active site residue]-

[1] Mitchell G.A., Robert M.-F., Hruz P.W., Wang S., Fontaine G., Behnke C.E., Mende-Mueller L.M., Schappert K., Lee C., Gibson K.M., Miziorko H.M. J. Biol. Chem. 268:4376-4381(1993).

[2] Hruz P.W., Narasimhan C., Miziorko H.M. Biochemistry 31:6842-6847(1992).

Alpha-isopropylmalate and homocitrate synthases signatures (HMGL2)

The following enzymes have been shown [1] to be functionally as well as evolutionary related: - Alpha-isopropylmalate synthase (EC <u>4.1.3.12</u>) which catalyzes the first step in the biosynthesis of leucine, the condensation of acetyl-CoA and alpha- ketoisovalerate to form 2-isopropylmalate synthase. - Homocitrate synthase (EC <u>4.1.3.21</u>) (gene nifV) which is involved in the biosynthesis of the iron-molybdenum cofactor of nitrogenase and catalyzes the condensation of acetyl-CoA and alpha-ketoglutarate into homocitrate. - Soybean late nodulin 56. - Methanococcus jannaschii hypothetical proteins MJ0503, MJ1195 and MJ1392. Two conserved regions were selected as signature patterns for these enzymes. The first region is located in the N-terminal section while the second region is located in the central section and contains two conserved histidine residues which could be implicated in the catalytic mechanism.

Consensus pattern: L-R-[DE]-G-x-Q-x(10)-K-

Consensus pattern: [LIVMFW]-x(2)-H-x-H-[DN]-D-x-G-x-[GAS]-x-[GASLI]-

[1] Wang S.-Z., Dean D.R., Chen J.-S., Johnson J.L. J. Bacteriol. 173:3041-3046(1991).

278. (HMG C0A synt) Hydroxymethylglutaryl-coenzyme A synthase active site Hydroxymethylglutaryl-coenzyme A synthase (EC <u>4.1.3.5</u>) (HMG-CoA synthase) catalyzes the condensation of acetyl-CoA with acetoacetyl-CoA to produce HMG- CoA and CoA [1].In vertebrates there are two isozymes located in different subcellular compartments: a cytosolic form which is the starting point of the mevalonate pathway which leads to cholesterol and other sterolic and isoprenoid compounds and a mitochondrial form responsible for ketone body biosynthesis. HMG-CoA is also found in other eukaryotes such as insect, plants and fungi. A cysteine is known to act as the catalytic nucleophile in the first step of the reaction,

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the acetylation of the enzyme by acetyl-CoA. The conserved region was used around this active site residue as a signature pattern.

Consensus pattern: N-x-[DN]-[IV]-E-G-[IV]-D-x(2)-N-A-C-[FY]-x-G [C is the active site residue]-

[1] Rokosz L.L., Boulton D.A., Butkiewicz E.A., Sanyal G., Cueto M.A., Lachance P.A., Hermes J.D. Arch. Biochem. Biophys. 312:1-13(1994).

279. HMG (high mobility group) box

280. HSF-type DNA-binding domain signature

Heat shock factor (HSF) is a DNA-binding protein that specifically binds heat shock promoter elements (HSE). HSE is a palindromic element rich with repetitive purine and pyrimidine motifs: 5'-nGAAnnTTCnnGAAnnTTCn-3'. HSF is expressed at normal temperatures but is activated by heat shock or chemical stressors [1,2]. The sequences of HSF from various species show extensive similarity in a region of about 90 amino acids, which has been shown [3] to bind DNA. Some other proteins also contain a HSF domain, these are: - Yeast SFL1, a protein involved in cell surface assembly and regulation of the gene related to flocculation (asexual cell aggregation) [4]. - Yeast transcription factor SKN7 (or BRY1 or POS9), which binds to the promoter elements SCB and MCB essential for the control of G1 cyclins expression [5]. - Yeast MGA1. - Yeast hypothetical protein YJR147w. A pattern from the most conserved part of the HSF DNA-binding domain was derived, its central region.

Consensus pattern: L-x(3)-[FY]-K-H-x-N-x-[STAN]-S-F-[LIVM]-R-Q-L-[NH]-x-Y-x-[FYW]-[RKH]-K-[LIVM]-

[1] Sorger P.K. <u>Cell 65:363-366(1991).</u>

[2] Mager W.H., Moradas Ferreira P. Biochem. J. 290:1-13(1993).

[3] Vuister G.W., Kim S.-J., Orosz A., Marquardt J., Wu C., Bax A. Nat. Struct. Biol. 1:605-613(1994).

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[4] Fujita A., Kikuchi Y., Kuhara S., Misumi Y., Matsumoto S., Kobayashi H. Gene 85:321-328(1989).

[5] Morgan B.A., Bouquin N., Merrill G.F., Johnston L.H. EMBO J. 14:5679-5689(1995).

281. Heat shock hsp20 proteins family profile

Prokaryotic and eukaryotic organisms respond to heat shock or other environmental stress by inducing the synthesis of proteins collectively known as heat-shock proteins (hsp) [1]. Amongst them is a family of proteins with an average molecular weight of 20 Kd, known as the hsp20 proteins [2 to 5]. These seem to act as chaperones that can protect other proteins against heat-induced denaturation and aggregation. Hsp20 proteins seem to form large heterooligomeric aggregates; their family is currently composed of the following members: - Vertebrate heat shock protein hsp27 (hsp25), induced by a variety of environmental stresses. - Drosophila heat shock proteins hsp22, hsp23, hsp26, hsp27, hsp67BA and BC. - Caenorhabditis elegans hsp16 multigene family. - Fungal HSP26 (budding yeast) and hsp30 (Neurospora crassa and Aspergillus Nidulans). - Plant small hsp's. Plants have four classes of hsp20: classes I and II which are cytoplasmic, class III which is chloroplastic and class IV which is found in the endomembrane. - Alpha-crystallin A and B chains. Alpha-crystallin is an abundant constituent of the eye lens of most vertebrate species. Its main function appears to be to maintain the correct refractive index of the lens. It is also found in other tissues where it seems to act as a chaperone [6]. - Schistosoma mansoni major egg antigen p40. Structurally, p40 is built of two tandem hsp20 domains. - A variety of prokaryotic proteins: ibpA and ibpB from Escherichia coli, hsp18 from Clostridium acetobutylicum, spore protein SP21 (hspA) from Stigmatella aurantiaca, Mycobacterium leprae 18 Kd antigen and Mycobacterium tuberculosis 14 Kd antigen. - Methanococcus jannaschii hypothetical protein MJ0285.Structurally, this family is characterized by the presence of a conserved C-terminal domain of about 100 residues. The profile developed to detect members of the hsp20 family is based on an alignment of this domain.

-Sequences known to belong to this class detected by the profile: ALL.

[1] Lindquist S., Craig E.A. Annu. Rev. Genet. 22:631-677(1988).[2] de Jong W.W., Leunissen J.A.M., Voorter C.E.M. Mol. Biol. Evol. 10:103-126(1993).[3] Caspers G.J., Leunissen J.A.M., de Jong W.W. J. Mol. Evol. 40:238-248(1995).[4] Jaenicke R., Creighton T.E. Curr. Biol. 3:234-235(1993).[5] Jakob U., Buchner J. Trends Biochem. Sci. 19:205-

211(1994).[6] Groenen P.J.T.A., Merck K.B., de Jong W.W., Bloemendal H. Eur. J. Biochem. 225:1-9(1994).

282. Heat shock hsp70 proteins family signatures

Prokaryotic and eukaryotic organisms respond to heat shock or other environmental stress by the induction of the synthesis of proteins collectively known as heat-shock proteins (hsp) [1]. Amongst them is a family of proteins with an average molecular weight of 70 Kd, known as the hsp70proteins [2,3,4]. In most species, there are many proteins that belong to the hsp70 family. Some of them are expressed under unstressed conditions. Hsp70proteins can be found in different cellular compartments (nuclear, cytosolic, mitochondrial, endoplasmic reticulum, etc.). Some of the hsp70 family proteinsare listed below: - In Escherichia coli and other bacteria, the main hsp70 protein is known as the dnaK protein. A second protein, hscA, has been recently discovered. dnaK is also found in the chloroplast genome of red algae. - In yeast, at least ten hsp70 proteins are known to exist: SSA1 to SSA4, SSB1, SSB2, SSC1, SSD1 (KAR2), SSE1 (MSI3) and SSE2. - In Drosophila, there are at least eight different hsp70 proteins: HSP70, HSP68, and HSC-1 to HSC-6. - In mammals, there are at least eight different proteins: HSPA1 to HSPA6, HSC70, and GRP78 (also known as the immunoglobulin heavy chain binding protein (BiP)). - In the sugar beet yellow virus (SBYV), a hsp70 homolog has been shown [5] to exist. - In archaebacteria, hsp70 proteins are also present [6].All proteins belonging to the hsp70 family bind ATP. A variety of functions has been postulated for hsp70 proteins. It now appears [7] that some hsp70proteins play an important role in the transport of proteins across membranes. They also seem to be involved in protein folding and in the assembly/disassembly of protein complexes [8]. Three signature patterns for the hsp70 family of proteins were derived; the first centered on a conserved pentapeptide found in the N-terminal section of these proteins; the two others on conserved regions located in the central part of the sequence.

Consensus pattern: [IV]-D-L-G-T-[ST]-x-[SC] -

Consensus pattern: [LIVMF]-[LIVMFY]-[DN]-[LIVMFS]-G-[GSH]-[GS]-[AST]-x(3)- [ST]-[LIVM]-[LIVMFC]-

Consensus pattern: [LIVMY]-x-[LIVMF]-x-G-G-x-[ST]-x-[LIVM]-P-x-[LIVM]-x-[DEQKRSTA]-

[1] Lindquist S., Craig E.A. Annu. Rev. Genet. 22:631-677(1988).

[2] Pelham H.R.B. Cell 46:959-961(1986).

[3] Pelham H.R.B. Nature 332:776-77(1988).[4] Craig E.A. BioEssays 11:48-52(1989).

[5] Agranovsky A.A., Boyko V.P., Karasev A.V., Koonin E.V., Dolja V.V. J. Mol. Biol. 217:603-610(1991).

[6] Gupta R.S., Singh B. J. Bacteriol. 174:4594-4605(1992).

[7] Deshaies R.J., Koch B.D., Schekmam R. Trends Biochem. Sci. 13:384-388(1988).

[8] Craig E.A., Gross C.A. Trends Biochem. Sci. 16:135-140(1991).

283. Heat shock hsp90 proteins family signature

Prokaryotic and eukaryotic organisms respond to heat shock or other environmental stress by the induction of the synthesis of proteins collectively known as heat-shock proteins (hsp) [1]. Amongst them is a family of proteins, with an average molecular weight of 90 Kd, known as the hsp90proteins. Proteins known to belong to this family are: - Escherichia coli and other bacteria heat shock protein c62.5 (gene htpG). - Vertebrate hsp 90-alpha (hsp 86) and hsp 90-beta (hsp 84). - Drosophila hsp 82 (hsp 83). - Trypanosoma cruzi hsp 85. - Plants Hsp82 or Hsp83. - Yeast and other fungi HSC82, and HSP82. - The endoplasmic reticulum protein 'endoplasmin' (also known as Erp99 in mouse, GRP94 in hamster, and hsp 108 in chicken).The exact function of hsp90 proteins is not yet known. In higher eukaryotes, hsp90 has been found associated with steroid hormone receptors, with tyrosine kinase oncogene products of several retroviruses, with eIF2alpha kinase, and with actin and tubulin. Hsp90 are probable chaperonins that possess ATPase activity [2,3].As a signature pattern for the hsp90 family of proteins, a highly conserved region found in the N-terminal part of these proteins was selected.

Consensus pattern: Y-x-[NQH]-K-[DE]-[IVA]-F-L-R-[ED] -

[1] Lindquist S., Craig E.A. Annu. Rev. Genet. 22:631-677(1988).

[2] Nadeau K., Das A., Walsh C.T. J. Biol. Chem. 268:1479-1487(1993).

[3] Jakob U., Buchner J. Trends Biochem. Sci. 19:205-211(1994).

284. Helix-turn-helix (HTH3)

This large family of DNA binding helix-turn helix proteins includes Cro Swiss:P03036 and CI Swiss:P03034.

285. Heme oxygenase signature

Heme oxygenase (EC 1.14.99.3) (HO) [1] is the microsomal enzyme that, in animals, carries out the oxidation of heme, it cleaves the heme ring at the alpha methene bridge to form biliverdin and carbon monoxide. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. In mammals there are three isozymes of heme oxygenase: HO-1 to HO-3. The first two isozymes differ in their tissue expression and their inducibility: HO-1 is highly inducible by its substrate heme and by various non-heme substances, while HO-2 is non-inducible. It has been suggested [2] that HO-2 could be implicated in the production of carbon monoxide in the brain where it is said to act as a neurotransmitter.In the genome of the chloroplast of red algae as well as in cyanobacteria, there is a heme oxygenase (gene pbsA) that is the key enzyme in the synthesis of the chromophoric part of the photosynthetic antennae [3]. An heme oxygenase is also present in the bacteria Corynebacterium diphtheriae (gene hmuO), where it is involved in the acquisition of iron from the host heme [4].There is, in the central section of these enzymes, a well conserved region centered on a histidine residue which is proposed to play a key role in binding the substrate heme at the active center of the enzyme. This region was used as a signature pattern.

Consensus pattern: L-[IV]-A-H-[STACH]-Y-[STV]-[RT]-Y-[LIVM]-G [H binds the heme] -

[1] Maines M.D. FASEB J. 2:2557-2568(1988).
[2] Barinaga M. Science 259:309-309(1993).
[3] Richaud C., Zabulon G. Proc. Natl. Acad. Sci. U.S.A. 94:11736-11741(1997).
[4] Schmitt M.P. J. Bacteriol. 179:838-845(1997).

286. Hepatitis core antigen.

The core antigen of hepatitis viruses possesses a carboxyl terminus rich in arginine. On this basis it was predicted that the core antigen would bind DNA [1]. There is some experimental evidence to support this [2].

[1] Pasek M, Goto T, Gilbert W, Zink B, Schaller H, Mckay P, Leadbetter G, Murray K; Nature 1979;282:575-579. [2] Gallina A, Bonelli F, Zentilin L, Rindi G, Muttini M, Milanesi G; J Virol 1989;63:4645-4652.

287. Histidine biosynthesis protein

Proteins involved in steps 4 and 6 of the histidine biosynthesis pathway are contained in this family. Histidine is formed by several complex and distinct biochemical reactions catalysed by eight enzymes. The enzymes in this Pfam entry are called His6 and His7 in eukaryotes and HisA and HisF in prokaryotes.

[1] Fani R, Tamburini E, Mori E, Lazcano A, Lio P, Barberio C, Casalone E, Cavalieri D, Perito B, Polsinelli M, Gene 1997;197:9-17. [2] Fani R, Lio P, Chiarelli I, Bazzicalupo M, J Mol Evol 1994;38:489-495.

288. Histone deacetylase family

Histones can be reversibly acetylated on several lysine residues. Regulation of transcription is caused in part by this mechanism. Histone deacetylases catalyse the removal of the acetyl group. Histone deacetylases are related to other proteins [1].

Leipe DD, Landsman D, Nucleic Acids Res 1997;25:3693-3697.

289. Histidinol dehydrogenase signature

Histidinol dehydrogenase (EC 1.1.1.23) (HDH) catalyzes the terminal step in the biosynthesis of histidine in bacteria, fungi, and plants, the four-electron oxidation of L-histidinol to histidine.In bacteria HDH is a single chain polypeptide; in fungi it is the C-terminal domain of a multifunctional enzyme which catalyzes three different steps of histidine biosynthesis;

and in plants it is expressed as nuclear encoded protein precursor which is exported to the chloroplast [1].As a signature pattern a highly conserved region located in the central part of HDH was selected. This region does not correspond to the part of the enzyme that, in most, but not all HDH sequences contains a cysteine residue which, in Salmonella typhimurium, has been said [2] to be important for the catalytic activity of the enzyme.

Consensus pattern: I-D-x(2)-A-G-P-[ST]-E-[LIVS]-[LIVMA](3)-[AC]-x(3)-A-x(4)- [LIVM]-[AV]-[SACL]-[DE]-[LIVMFC]-[LIVM]-[SA]-x(2)-E-H-

[1] Nagai A., Ward E., Beck J., Tada S., Chang J.-Y., Scheidegger A., Ryals J. Proc. Natl. Acad. Sci. U.S.A. 88:4133-4137(1991).

[2] Grubmeyer C.T., Gray W.R. Biochemistry 25:4778-4784(1986).

290. Homoserine dehydrogenase signature

Homoserine dehydrogenase (EC 1.1.1.3) (HDh) [1,2] catalyzes NAD-dependent reduction of aspartate beta-semialdehyde into homoserine. This reaction is the third step in a pathway leading from aspartate to homoserine. The latter participates in the biosynthesis of threonine and then isoleucine as well as in that of methionine. HDh is found either as a single chain protein as in some bacteria and yeast, or as a bifunctional enzyme consisting of an N-terminal aspartokinase domain and a C-terminal HDh domain as in bacteria such as Escherichia coli and in plants. As a signature pattern, the best conserved region of Hdh has been selected. This is a segment of 23 to 24 residues located in the central section and that contains two conserved aspartate residues.

Consensus pattern: A-x(3)-G-[LIVMFY]-[STAG]-x(2,3)-[DNS]-P-x(2)-D-[LIVM]-x-G- x-D-x(3)-K-

[1] Thomas D., Barbey R., Surdin-Kerjan Y. FEBS Lett. 323:289-293(1993).

[2] Cami B., Clepet C., Patte J.-C. Biochimie 75:487-495(1993).

291. haloacid dehalogenase-like hydrolase

This family is structurally different from the alpha/ beta hydrolase family (abhydrolase). This family includes L-2-haloacid dehalogenase, epoxide hydrolases and phosphatases. The structure of the family consists of two domains. One is an inserted four helix bundle, which is the least well conserved region of the alignment, between residues 16 and 96 of Swiss:P24069. The rest of the fold is composed of the core alpha/beta domain.

[1] Hisano T, Hata Y, Fujii T, Liu JQ, Kurihara T, Esaki N, Soda K, J Biol Chem 1996; 271:20322-20330.

292. DEAD and DEAH box families ATP-dependent helicases signatures (helicase_C)

A number of eukaryotic and prokaryotic proteins have been characterized [1,2,3] on the basis of their structural similarity. They all seem to be involved in ATP-dependent, nucleic-acid unwinding. Proteins currently known to belong to this family are: - Initiation factor eIF-4A. Found in eukaryotes, this protein is a subunit of a high molecular weight complex involved in 5'cap recognition and the binding of mRNA to ribosomes. It is an ATP-dependent RNA-helicase. - PRP5 and PRP28. These yeast proteins are involved in various ATP-requiring steps of the pre-mRNA splicing process. - Pl10, a mouse protein expressed specifically during spermatogenesis. - An3, a Xenopus putative RNA helicase, closely related to Pl10. - SPP81/DED1 and DBP1, two yeast proteins probably involved in pre-mRNA splicing and related to Pl10. - Caenorhabditis elegans helicase glh-1. - MSS116, a yeast protein required for mitochondrial splicing. - SPB4, a yeast protein involved in the maturation of 25S ribosomal RNA. - p68, a human nuclear antigen. p68 has ATPase and DNA-helicase activities in vitro. It is involved in cell growth and division. - Rm62 (p62), a Drosophila putative RNA helicase related to p68. - DBP2, a yeast protein related to p68. - DHH1, a yeast protein. - DRS1, a yeast protein involved in ribosome assembly. - MAK5, a yeast protein involved in maintenance of dsRNA killer plasmid. - ROK1, a yeast protein. - ste13, a fission yeast protein. - Vasa, a Drosophila protein important for oocyte formation and specification of embryonic posterior structures. - Me31B, a Drosophila maternally expressed protein of unknown function. - dbpA, an Escherichia coli putative RNA helicase. - deaD, an Escherichia coli putative RNA helicase which can suppress a mutation in the rpsB gene for ribosomal protein S2. - rhlB, an Escherichia coli putative RNA helicase. - rhlE, an Escherichia coli putative RNA helicase. - srmB, an Escherichia coli protein that shows RNA-dependent ATPase activity. It probably interacts with 23S ribosomal RNA. - Caenorhabditis elegans

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hypothetical proteins T26G10.1, ZK512.2 and ZK686.2. - Yeast hypothetical protein YHR065c. - Yeast hypothetical protein YHR169w. - Fission yeast hypothetical protein SpAC31A2.07c. - Bacillus subtilis hypothetical protein yxiN. All these proteins share a number of conserved sequence motifs. Some of them are specific to this family while others are shared by other ATP-binding proteins or by proteins belonging to the helicases `superfamily' [4,E1]. One of these motifs, called the 'D-E-A-D-box', represents a special version of the B motif of ATP-binding proteins. Some other proteins belong to a subfamily which have His instead of the second Asp and are thus said to be 'D-E-A-H-box' proteins [3,5,6,E1]. Proteins currently known to belong to this subfamily are: - PRP2, PRP16, PRP22 and PRP43. These yeast proteins are all involved in various ATP-requiring steps of the pre-mRNA splicing process. - Fission yeast prh1, which my be involved in pre-mRNA splicing. - Male-less (mle), a Drosophila protein required in males, for dosage compensation of X chromosome linked genes. - RAD3 from yeast. RAD3 is a DNA helicase involved in excision repair of DNA damaged by UV light, bulky adducts or cross-linking agents. Fission yeast rad15 (rhp3) and mammalian DNA excision repair protein XPD (ERCC-2) are the homologs of RAD3. - Yeast CHL1 (or CTF1), which is important for chromosome transmission and normal cell cycle progression in G(2)/M. - Yeast TPS1. - Yeast hypothetical protein YKL078w. - Caenorhabditis elegans hypothetical proteins C06E1.10 and K03H1.2. - Poxviruses' early transcription factor 70 Kd subunit which acts with RNA polymerase to initiate transcription from early gene promoters. - I8, a putative vaccinia virus helicase. - hrpA, an Escherichia coli putative RNA helicase. Signature patterns were developed for both subfamilies.

Consensus pattern: [LIVMF](2)-D-E-A-D-[RKEN]-x-[LIVMFYGSTN]-
Consensus pattern: [GSAH]-x-[LIVMF](3)-D-E-[ALIV]-H-[NECR] -
Note: proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop) (see the relevant entry <PDOC00017

[1] Schmid S.R., Linder P. Mol. Microbiol. 6:283-292(1992).
[2] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).
[3] Wassarman D.A., Steitz J.A. Nature 349:463-464(1991).
[4] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).

09595256.071502

[5] Harosh I., Deschavanne P. Nucleic Acids Res. 19:6331-6331(1991).

[6] Koonin E.V., Senkevich T.G. J. Gen. Virol. 73:989-993(1992).

293. Heme-binding domain in cytochrome b5 and oxidoreductases (heme_1)

Cytochrome b5 is a membrane-bound hemo protein which acts as an electron carrier for several membrane-bound oxygenases [1]. There are two homologous forms of b5, one found in microsomes and one found in the outer membrane of mitochondria. Two conserved histidine residues serve as axial ligands for the heme group. The structure of a number of oxidoreductases consists of the juxtaposition of a heme-binding domain homologous to that of b5 and either a flavodehydrogenase or a molybdopterin domain. These enzymes are:

- Lactate dehydrogenase (EC 1.1.2.3) [2], an enzyme that consists of a flavodehydrogenase domain and a heme-binding domain called cytochrome b2.
- Nitrate reductase (EC 1.6.6.1), a key enzyme involved in the first step of nitrate assimilation in plants, fungi and bacteria [3,4]. Consists of a molybdopterin domain (see <PDOC00484>), a heme-binding domain called cytochrome b557, as well as a cytochrome reductase domain.
- Sulfite oxidase (EC 1.8.3.1) [5], which catalyzes the terminal reaction in the oxidative degradation of sulfur-containing amino acids. Also consists of a molybdopterin domain and a heme-binding domain.

This family of proteins also includes:

- TU-36B, a Drosophila muscle protein of unknown function [6].
- Fission yeast hypothetical protein SpAC1F12.10c.
- Yeast hypothetical protein YMR073c.
- Yeast hypothetical protein YMR272c.

A segment was used which includes the first of the two histidine heme ligands, as a signature pattern for the heme-binding domain of cytochrome b5 family.

Consensus pattern: [FY]-[LIVMK]-x(2)-H-P-[GA]-G [H is a heme axial ligand]-

[1] Ozols J. Biochim. Biophys. Acta 997:121-130(1989).

[2] Guiard B. EMBO J. 4:3265-3272(1985).

[3] Calza R., Huttner E., Vincentz M., Rouze P., Galangau F., Vaucheret H., Cherel I., Meyer C., Kronenberger J., Caboche M. Mol. Gen. Genet. 209:552-562(1987).

[4] Crawford N.M., Smith M., Bellissimo D., Davis R.W. Proc. Natl. Acad. Sci. U.S.A. 85:5006-5010(1988).

[5] Guiard B., Lederer F. Eur. J. Biochem. 100:441-453(1979).

[6] Levin R.J., Boychuk P.L., Croniger C.M., Kazzaz J.A., Rozek C.E. Nucleic Acids Res. 17:6349-6367(1989).

294. Hexapeptide-repeat containing-transferases signature

On the basis of sequence similarity, a number of transferases have been proposed [1,2,3,4] to belong to a single family. These proteins are: - Serine acetyltransferase (EC 2.3.1.30) (SAT) (gene cysE), an enzyme involved in cysteine biosynthesis. - Azotobacter chroococcum nitrogen fixation protein nifP. NifP is most probably a SAT involved in the optimization of nitrogenase activity. - Escherichia coli thiogalactoside acetyltransferase (EC 2.3.1.18) (gene lacA), an enzyme involved in the biosynthesis of lactose. - UDP-N-acetylglucosamine acyltransferase (EC 2.3.1.129) (gene lpxA), an enzyme involved in the biosynthesis of lipid A, a phosphorylated glycolipid that anchors the lipopolysaccharide to the outer membrane of the cell. - UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase (EC 2.3.1.-) (gene lpxD or firA), which is also involved in the biosynthesis of lipid A. - Chloramphenicol acetyltransferase (CAT) (EC 2.3.1.28) from Agrobacterium tumefaciens, Bacillus sphaericus, Escherichia coli plasmid IncFII NR79, Pseudomonas aeruginosa, Staphylococcus aureus plasmid pIP630. These CAT are not evolutionary related to the main family of CAT (see <PDOC00093>). - Rhizobium nodulation protein nodL. NodL is an acetyltransferase involved in the O-acetylation of Nod factors. - Bacterial maltose O-acetyltransferase (EC 2.3.1.79). - Bacterial tetrahydrodipicolinate N-succinyltransferase (EC 2.3.1.117) (gene dapD) which catalyzes the fourth step in the biosynthesis of diaminopimelate and lysine from aspartate semialdehyde. - Bacterial N-acetylglucosamine-1-phosphate uridyltransferase (EC 2.7.7.23) (gene glmU or gcaD or tms), an enzyme involved in peptidoglycan and lipopolysaccharide biosynthesis. - Staphylococcus aureus protein capG which is involved in biosynthesis of type 1 capsular polysaccharide. - Yeast hypothetical protein YJL218w, which is highly similar to Escherichia coli lacA. - Fission yeast hypothetical protein SpAC18B11.09c. - Methanococcus jannaschii hypothetical protein MJ1064.These proteins have been shown [3,4] to contain a repeat structure composed of tandem repeats of a [LIV]-G-x(4) hexapeptide which, in the tertiary structure of lpxA [5], has been shown to form a left-handed parallel beta helix. Our signature pattern is based on a fourfold repeat of this hexapeptide.

Consensus pattern: [LIV]-[GAED]-x(2)-[STAV]-x-[LIV]-x(3)-[LIVAC]-x-[LIV]- [GAED]-x(2)-[STAVR]-x-[LIV]-[GAED]-x(2)-[STAV]-x-[LIV]- x(3)-[LIV]-

[1] Downie J.A. Mol. Microbiol. 3:1649-1651(1989).
[2] Parent R., Roy P.H. J. Bacteriol. 174:2891-2897(1992).
[3] Vaara M. FEMS Microbiol. Lett. 97:249-254(1992).
[4] Vuorio R., Haerkonen T., Tolvanen M., Vaara M. FEBS Lett. 337:289-292(1994).
[5] Raetz C.R.H., Roderick S.L. Science 270:997-1000(1995).

09525325.052600

295. Hexokinases signature. Hexokinase (EC 2.7.1.1) [1,2] is an important glycolytic enzyme that catalyzes the phosphorylation of keto- and aldohexoses (e.g. glucose, mannose and fructose) using MgATP as the phosphoryl donor. In vertebrates there are four major isoenzymes, commonly referred as types I,II, III and IV. Type IV hexokinase, which is often incorrectly designated glucokinase [3], is only expressed in liver and pancreatic beta-cells and plays an important role in modulating insulin secretion; it is a protein of a molecular mass of about 50 Kd. Hexokinases of types I to III, which have low Km values for glucose, have a molecular mass of about 100 Kd. Structurally they consist of a very small N-terminal hydrophobic membrane-binding domain followed by two highly similar domains of 450 residues. The first domain has lost its catalytic activity and has evolved into a regulatory domain. In yeast there are three different isozymes: hexokinase PI (gene HXK1), PII(gene HXKB), and glucokinase (gene GLK1). All three proteins have a molecular mass of about 50 Kd. All these enzymes contain one (or two in the case of types I to III isozymes)strongly conserved region which has been shown [4] to be involved in substrate binding. A pattern from that region has been derived

Consensus pattern: [LIVM]-G-F-[TN]-F-S-[FY]-P-x(5)-[LIVM]-[DNST]-x(3)-[LIVM]- x(2)-W-T-K-x-[LF]-

[1] Middleton R.J. Biochem. Soc. Trans. 18:180-183(1990).[2] Griffin L.D., Gelb B.D., Wheeler D.A., Davison D., Adams V., McCabe E.R. Genomics 11:1014-1024(1991).[3] Cornish-Bowden A., Luz Cardenas M. Trends Biochem. Sci. 16:281-282(1991).[4] Schirch D.M., Wilson J.E. Arch. Biochem. Biophys. 254:385-396(1987).

296. Histone H2A signature (his1)

Histone H2A is one of the four histones, along with H2B, H3 and H4, which forms the eukaryotic nucleosome core. Using alignments of histone H2Asequences [1,2,E1] as a signature pattern, a conserved region in the N-terminal part of H2A. This region is conserved both in classical S-phase regulated H2A's and in variant histone H2A's which are synthesized throughout the cell cycle.

09595329.061500

Consensus pattern: [AC]-G-L-x-F-P-V-

[1] Wells D.E., Brown D. Nucleic Acids Res. 19:2173-2188(1991).
[2] Thatcher T.H., Gorovsky M.A. Nucleic Acids Res. 22:174-179(1994).

Histone H4 signature (his2)

Histone H4 is one of the four histones, along with H2A, H2B and H3, which forms the eukaryotic nucleosome core. Along with H3, it plays a central role in nucleosome formation. The sequence of histone H4 has remained almost invariant in more then 2 billion years of evolution [1,E1]. The region used as a signature pattern is a pentapeptide found in positions 14 to 18 of all H4sequences. It contains a lysine residue which is often acetylated [2] and a histidine residue which is implicated in DNA-binding [3].

Consensus pattern: G-A-K-R-H-

[1] Thatcher T.H., Gorovsky M.A. Nucleic Acids Res. 22:174-179(1994).
[2] Doenecke D., Gallwitz D. Mol. Cell. Biochem. 44:113-128(1982).
[3] Ebralidse K.K., Grachev S.A., Mirzabekov A.D. Nature 331:365-367(1988).

Histone H3 signatures (his3)

Histone H3 is one of the four histones, along with H2A, H2B and H4, which forms the eukaryotic nucleosome core. It is a highly conserved protein of 135 amino acid residues [1,2,E1].The following proteins have been found to contain a C-terminal H3-like domain: - Mammalian centromeric protein CENP-A [3]. Could act as a core histone necessary for the assembly of centromeres. - Yeast chromatin-associated protein CSE4 [4]. - Caenorhabditis elegans chromosome III encodes two highly related proteins (F54C8.2 and F58A4.3) whose C-terminal section is evolutionary related to the last 100 residues of H3. The function of these proteins is not yet known. Two signature patterns were developed, The first one corresponds to a perfectly conserved heptapeptide in the N-terminal part of H3. The second one is derived from a conserved region in the central section of H3.

Consensus pattern: K-A-P-R-K-Q-L-

Consensus pattern: P-F-x-[RA]-L-[VA]-[KRQ]-[DEG]-[IV]-

[1] Wells D.E., Brown D. Nucleic Acids Res. 19:2173-2188(1991).
[2] Thatcher T.H., Gorovsky M.A. Nucleic Acids Res. 22:174-179(1994).
[3] Sullivan K.F., Hechenberger M., Masri K. J. Cell Biol. 127:581-592(1994).
[4] Stoler S., Keith K.C., Curnick K.E., Fitzgerald-Hayes M. Genes Dev. 9:573-586(1995).

Histone H2B signature (his4)

Histone H2B is one of the four histones, along with H2A, H3 and H4, which forms the eukaryotic nucleosome core. Using alignments of histone H2Bsequences [1,2,E1], a conserved region was selected in the C-terminal part ofH2B.

Consensus pattern: [KR]-E-[LIVM]-[EQ]-T-x(2)-[KR]-x-[LIVM](2)-x-[PAG]-[DE]-L- x-[KR]-H-A-[LIVM]-[STA]-E-G-

[1] Wells D.E., Brown D. Nucleic Acids Res. 19:2173-2188(1991).
[2] Thatcher T.H., Gorovsky M.A. Nucleic Acids Res. 22:174-179(1994).

297. 'Homeobox' domain signature and profile (home1)

The 'homeobox' is a protein domain of 60 amino acids [1 to 5,E1] first identified in a number of Drosophila homeotic and segmentation proteins. It has since been found to be extremely well conserved in many other animals, including vertebrates. This domain binds DNA through a helix-turn-helix type of structure. Some of the proteins which contain a homeobox domain play an important role in development. Most of these proteins are known to be sequence specific DNA-binding transcription factors. The homeobox domain has also been found to be very similar to a region of the yeast mating type proteins. These are sequence-specific DNA-binding proteins that act as master switches in yeast differentiation by controlling gene expression in a cell type-specific fashion. A schematic representation of the homeobox domain is shown below. The helix-turn-helix region is shown by the symbols 'H' (for helix), and 't' (for turn).

xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxHHHHHHHtttHHHHHHHHHxxxxxxxxxx | | | | | | | 1 10 20 30 40 50 60 The pattern to detect homeobox sequences that was developed is 24 residues long and spans positions 34 to 57 of the homeobox domain.

Consensus pattern: [LIVMFYG]-[ASLVR]-x(2)-[LIVMSTACN]-x-[LIVM]-x(4)-[LIV]-[RKNQESTAIY]-[LIVFSTNKH]-W-[FYVC]-x-[NDQTAH]-x(5)- [RKNAIMW] -

[1] Gehring W.J. (In) Guidebook to the homebox genes, Duboule D., Ed., pp1-10, Oxford University Press, Oxford, (1994).

[2] Buerglin T.R. (In) Guidebook to the homebox genes, Duboule D., Ed., pp25-72, Oxford University Press, Oxford, (1994).

[3] Gehring W.J. Trends Biochem. Sci. 17:277-280(1992).

[4] Gehring W.J., Hiromi Y. Annu. Rev. Genet. 20:147-173(1986).

[5] Schofield P.N. Trends Neurosci. 10:3-6(1987).

'Homeobox' antennapedia-type protein signature (home2)

The homeotic Hox proteins are sequence-specific transcription factors. They are part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior (A-P) axis [1]. The hox proteins contain a 'homeobox' domain. In Drosophila and other insects, there are eight different Hox genes that are encoded in two gene complexes, ANT-C and BX-C. In vertebrates there are 38 genes organized in four complexes. In six of the eight Drosophila Hox genes the homeobox domain is highly similar and a conserved hexapeptide is found five to sixteen amino acids upstream of the homeobox domain. The six Drosophila proteins that belong to this group are antennapedia (Antp), abdominal-A (abd-A), deformed (Dfd), proboscipedia (pb),sex combs reduced (scr) and ultrabithorax (ubx) and are collectively known as the 'antennapedia' subfamily. In vertebrates the corresponding Hox genes are known [2] as Hox-A2, A3, A4,A5, A6, A7, Hox-B1, B2, B3, B4, B5, B6, B7, B8, Hox-C4, C5, C6, C8, Hox-D1,D3, D4 and D8.Caenorhabditis elegans lin-39 and mab-5 are also members of the 'antennapedia' subfamily. As a signature pattern for this subfamily of homeobox proteins, the conserved hexapeptide was used.

Consensus pattern: [LIVMFE]-[FY]-P-W-M-[KRQTA]-

[1] McGinnis W., Krumlauf R. <u>Cell 68:283-302(1992).</u>
[2] Scott M.P. <u>Cell 71:551-553(1992).</u>

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'Homeobox' engrailed-type protein signature (home3)

Most proteins which contain a 'homeobox' domain can be classified [1,2], on the basis of their sequence characteristics, in three subfamilies: engrailed, antennapedia and paired. Proteins currently known to belong to the engrailed subfamily are: - Drosophila segmentation polarity protein engrailed (en) which specifies the body segmentation pattern and is required for the development of the central nervous system. - Drosophila invected protein (inv). - Silk moth proteins engrailed and invected, which may be involved in the compartmentalization of the silk gland. - Honeybee E30 and E60. - Grasshopper (Schistocerca americana) G-En. - Mammalian and birds En-1 and En-2. - Zebrafish Eng-1, -2 and -3. - Sea urchin (Tripneusteas gratilla) SU-HB-en. - Leech (Helobdella triserialis) Ht-En. - Caenorhabditis elegans ceh-16.Engrailed homeobox proteins are characterized by the presence of a conserved region of some 20 amino-acid residues located at the C-terminal of the 'homeobox' domain. As a signature pattern for this subfamily of proteins, a stretch of eight perfectly conserved residues in this region was used.

Consensus pattern: L-M-A-[EQ]-G-L-Y-N-

[1] Scott M.P., Tamkun J.W., Hartzell G.W. III Biochim. Biophys. Acta 989:25-48(1989).
[2] Gehring W.J. Science 236:1245-1252(1987).

298. Isocitrate lyase signature (ICL)

Isocitrate lyase (EC 4.1.3.1) [1,2] is an enzyme that catalyzes the conversion of isocitrate to succinate and glyoxylate. This is the first step in the glyoxylate bypass, an alternative to the tricarboxylic acid cycle in bacteria, fungi and plants. A cysteine, a histidine and a glutamate or aspartate have been found to be important for the enzyme's catalytic activity. Only one cysteine residue is conserved between the sequences of the fungal, plant and bacterial enzymes; it is located in the middle of a conserved hexapeptide that can be used as a signature pattern for this type of enzyme.

Consensus pattern: K-[KR]-C-G-H-[LMQ] [C is a putative active site residue]-

[1] Beeching J.R. Protein Seq. Data Anal. 2:463-466(1989).
[2] Atomi H., Ueda M., Hikida M., Hishida T., Teranishi Y., Tanaka A. J. Biochem. 107:262-266(1990).

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299. Initiation factor 2 subunit

This family includes initiation factor 2B alpha, beta and delta subunits from eukaryotes, related proteins from archaebacteria and IF-2 from prokaryotes. Initiation factor 2 binds to Met-tRNA, GTP and the small ribosomal subunit.

[1] Kyrpides NC, Woese CR, Proc Natl Acad Sci U S A 1998;95:3726-3730.

300. Initiation factor 3 signature

Initiation factor 3 (IF-3) (gene infC) [1] is one of the three factors required for the initiation of protein biosynthesis in bacteria. IF-3 is thought to function as a fidelity factor during the assembly of the ternary initiation complex which consist of the 30S ribosomal subunit, the initiator tRNA and the messenger RNA. IF-3 binds to the 30S ribosomal subunit; it is a basic protein of 141 to 212 residues. The chloroplast initiation factor IF-3(chl) is a protein that enhances the poly(A,U,G)-dependent binding of the initiator tRNA to chloroplast ribosomal30s subunits. In its mature form it is a protein of about 400 residues whose central section is evolutionary related to the sequence of bacterial IF-3 [2].As a signature pattern a highly conserved region was selected located in the central section of bacterial IF-3 and of IF-3(chl).

Consensus pattern: [KR]-[LIVM](2)-[DN]-[FY]-[GSN]-[KR]-[LIVMFYS]-x-[FY]-[DEQTH]-x(2)-[KRQ]-

[1] Liveris D., Schwartz J.J., Geertman R., Schwartz I. FEMS Microbiol. Lett. 112:211-216(1993).
[2] Lin Q., Ma L., Burkhart W., Spremulli L.L. J. Biol. Chem. 269:9436-9444(1994).

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301. Imidazoleglycerol-phosphate dehydratase signatures (IGPD)
Imidazoleglycerol-phosphate dehydratase (EC 4.2.1.19) is the enzyme that catalyzes the seventh step in the biosynthesis of histidine in bacteria, fungi and plants. In most organisms it is a monofunctional protein of about 22 to29 Kd. In some bacteria such as Escherichia coli it is the C-terminal domain of a bifunctional protein that include a histidinol-phosphatase domain [1]. Two signature patterns were developed that each include two consecutive histidine residues.

Consensus pattern: [LIVMY]-[DE]-x-H-H-x(2)-E-x(2)-[GCA]-[LIVM]-[STAC]-[LIVM]-
Consensus pattern: G-x-[DN]-x-H-H-x(2)-E-[STAGC]-x-[FY]-K -

[1] Carlomagno M.S., Chiariotti L., Alifano P., Nappo A.G., Bruni C.B. J. Mol. Biol. 203:585-606(1988).

302. Indole-3-glycerol phosphate synthase signature (IGPS)
Indole-3-glycerol phosphate synthase (EC 4.1.1.48) (IGPS) catalyzes the fourth step in the biosynthesis of tryptophan: the ring closure of 1-(2-carboxy-phenylamino)-1-deoxyribulose into indol-3-glycerol-phosphate.In some bacteria, IGPS is a single chain enzyme. In others - such as Escherichia coli - it is the N-terminal domain of a bifunctional enzyme that also catalyzes N-(5'-phosphoribosyl)anthranilate isomerase (PRAI) activity, the third step of

tryptophan biosynthesis. In fungi, IGPS is the central domain of a trifunctional enzyme that also contains a PRAI C-terminal domain and a glutamine amidotransferase N-terminal domain. The N-terminal section of IGPS contains a highly conserved region which X-ray crystallography studies [1] have shown to be part of the active site cavity. This region was used as a signature pattern for IGPS.

Consensus pattern: [LIVMFY]-[LIVMC]-x-E-[LIVMFYC]-K-[KRSP]-[STAK]-S-P-[ST]-x(3)-[LIVMFYST]-

[1] Wilmanns M., Priestle J.P., Niermann T., Jansonius J.N. J. Mol. Biol. 223:477-507(1992).

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303. (IL2) Interleukin 2. 31 members

304. (ILVD EDD) Dihydroxy-acid and 6-phosphogluconate dehydratases. Two dehydratases have been shown [1] to be evolutionary related: - Dihydroxy-acid dehydratase (EC 4.2.1.9) (gene ilvD or ILV3) which catalyzes the fourth step in the biosynthesis of isoleucine and valine, the dehydratation of 2,3-dihydroxy-isovaleic acid into alpha-ketoisovaleric acid. - 6-phosphogluconate dehydratase (EC 4.2.1.12) (gene edd) which catalyzes the first step in the Entner-Doudoroff pathway, the dehydratation of 6-phospho- D-gluconate into 6-phospho-2-dehydro-3-deoxy-D-gluconate. - Escherichia coli hypothetical protein yjhG. Both enzymes are proteins of about 600 amino acid residues. Two highly conserved regions have been developed as signature patterns. The first pattern is located in the N-terminal part and contains a cysteine that could be involved in the binding of a 2Fe-2S iron-sulfur cluster [2]. The second pattern is located in the C-terminal half.

Consensus pattern: C-D-K-x(2)-P-[GA]-x(3)-[GA] [The C could be a 2Fe-2S ligand]
Consensus pattern: [SA]-L-[LIVM]-T-D-[GA]-R-[LIVMF]-S-[GA]-[GAV]-[ST]-

[1] Egan S.E., Fliege R., Tong S., Shibata A., Wolf R.E. Jr., Conway T. J. Bacteriol. 174:4638-4646(1992).[2] Velasco J.A., Cansado J., Pena M.C., Kawakami T., Laborda J., Notario V. Gene 137:179-185(1993).

305. IMP dehydrogenase / GMP reductase signature

IMP dehydrogenase (EC 1.1.1.205) (IMPDH) catalyzes the rate-limiting reaction of de novo GTP biosynthesis, the NAD-dependent reduction of IMP into XMP [1].Inhibition of IMP dehydrogenase activity results in the cessation of DNA synthesis. As IMP dehydrogenase is associated with cell proliferation, it is a possible target for cancer chemotherapy. Mammalian and bacterial IMPDHs are tetramers of identical chains. There are two IMP dehydrogenase isozymes in humans [2].GMP reductase (EC 1.6.6.8) catalyzes the irreversible and NADPH-dependent reductive deamination of GMP into IMP [3]. It converts nucleobase, nucleoside and nucleotide derivatives of G to A nucleotides, and maintains intracellular balance of A and G nucleotides. IMP dehydrogenase and GMP reductase share many regions of sequence similarity. One of these regions is centered on a cysteine residue thought [3] to be involved in binding IMP. This region was used as a signature pattern.

Consensus pattern: [LIVM]-[RK]-[LIVM]-G-[LIVM]-G-x-G-S-[LIVM]-C-x-T [C is the putative IMP-binding residue]-

[1] Collart F.R., Huberman E. J. Biol. Chem. 263:15769-15772(1988).
[2] Natsumeda Y., Ohno S., Kawasaki H., Konno Y., Weber G., Suzuki K. J. Biol. Chem. 265:5292-5295(1990).
[3] Andrews S.C., Guest J.R. Biochem. J. 255:35-43(1988).

306. (IPPc) Inositol polyphosphate phosphatase family, catalytic domain

[1] York JD, Ponder JW, Chen ZW, Mathews FS, Majerus PW; Biochemistry 1994;33:13164-13171. [2] Jefferson AB, Auethavekiat V, Pot DA, Williams LT, Majerus PW; J Biol Chem 1997;272:5983-5988. [3] Zhang X, Jefferson AB, Auethavekiat V, Majerus PW; Proc Natl Acad Sci U S A 1995;92:4853-4856. [4] York JD,

Majerus PW. Proc Natl Acad Sci U S A 1990;87:9548-9552. [5] Neuwald AF, York JD, Majerus PW;
FEBS Lett 1991;294:16-18.

307. IQ calmodulin-binding motif

[1] Xie X, Harrison DH, Schlichting I, Sweet RM, Kalabokis VN, Szent-Gyorgyi AG, Cohen C; Nature 1994;368:306-312.
[2] Rhoads AR, Friedberg F; FASEB J 1997;11:331-340.

308. Inosine-uridine preferring nucleoside hydrolasefamily signature (IU nuc hydro) Inosine-uridine preferring nucleoside hydrolase (EC 3.2.2.1) (IU-nucleosidehydrolase or IUNH) is an enzyme first identified in protozoan [1] that catalyzes the hydrolysis of all of the commonly occuring purine and pyrimidine nucleosides into ribose and the associated base, but has a preference for inosine and uridine as substrates. This enzyme is important for these parasitic organisms, which are deficient in de novo synthsis of purines, to salvage the host purine nucleosides. IUNH from Crithidia fasciculata has been sequenced and characterized, it is an homotetrameric enzyme of subunits of 34 Kd. An histidine has been shown to be important for the catalytic mechanism, it acts a proton donor to activate the hypoxanthine leaving group. IUNH is evolutionary related to a number of uncharacterized proteins from various biological sources, notably: - Escherichia coli hypothetical protein yaaF. - Escherichia coli hypothetical protein ybeK. - Escherichia coli hypothetical protein yeiK. - Fission yeast hypothetical protein SpAC17G8.02. - Yeast hypothetical protein YDR400w. - An hypothetical protein from the archaebacteria Desulfurolobus ambivalens. As a signature pattern for these proteins, a highly conserved region was selected located in the N-terminal extremity. This region contains four conserved aspartates that have been shown [2] to be located in the active site cavity.

Consensus pattern: D-x-D-[PT]-[GA]-x-D-D-[TAV]-[VI]-A -

[1] Gopaul D.N., Meyer S.L., Degano M., Sacchettini J.C., Schramm V.L. Biochemistry 35:5963-5970(1996).
[2] Degano M., Gopaul D.N., Scapin G., Schramm V.L., Sacchettini J.C. Biochemistry 35:5971-5981(1996).

309. (Insulinase)
Insulinase family, zinc-binding region signature
(aka Peptidase_M16)

A number of proteases dependent on divalent cations for their activity have been shown [1,2] to belong to one family, on the basis of sequence similarity. These enzymes are listed below.

- Insulinase (EC 3.4.24.56) (also known as insulysin or insulin-degrading enzyme or IDE), a cytoplasmic enzyme which seems to be involved in the cellular processing of insulin, glucagon and other small polypeptides.
- Escherichia coli protease III (EC 3.4.24.55) (pitrilysin) (gene ptr), a periplasmic enzyme that degrades small peptides.
- Mitochondrial processing peptidase (EC 3.4.24.64) (MPP). This enzyme removes the transit peptide from the precursor form of proteins imported from the cytoplasm across the mitochondrial inner membrane. It is composed of two nonidentical homologous subunits termed alpha and beta. The beta subunit seems to be catalytically active while the alpha subunit has probably lost its activity.
- Nardilysin (EC 3.4.24.61) (N-arginine dibasic convertase or NRD convertase) this mammalian enzyme cleaves peptide substrates on the N-terminus of Arg residues in dibasic stretches.
- Klebsiella pneumoniae protein pqqF. This protein is required for the biosynthesis of the coenzyme pyrrolo-quinoline-quinone (PQQ). It is thought to be protease that cleaves peptide bonds in a small peptide (gene pqqA) thus providing the glutamate and tyrosine residues necessary for the synthesis of PQQ.
- Yeast protein AXL1, which is involved in axial budding [3].
- Eimeria bovis sporozoite developmental protein.

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- Escherichia coli hypothetical protein yddC and HI1368, the corresponding Haemophilus influenzae protein.
- Bacillus subtilis hypothetical protein ymxG.
- Caenorhabditis elegans hypothetical proteins C28F5.4 and F56D2.1.

It should be noted that in addition to the above enzymes, this family also includes the core proteins I and II of the mitochondrial bc1 complex (also called cytochrome c reductase or complex III), but the situation as to the activity or lack of activity of these subunits is quite complex:

- In mammals and yeast, core proteins I and II lack enzymatic activity.
- In Neurospora crassa and in potato core protein I is equivalent to the beta subunit of MPP.
- In Euglena gracilis, core protein I seems to be active, while subunit II is inactive.

These proteins do not share many regions of sequence similarity; the most noticeable is in the N-terminal section. This region includes a conserved histidine followed, two residues later by a glutamate and another histidine. In pitrilysin, it has been shown [4] that this H-x-x-E-H motif is involved in enzyme activity; the two histidines bind zinc and the glutamate is necessary for catalytic activity. Non active members of this family have lost from one to three of these active site residues. We developed a signature pattern that detect active members of this family as well as some inactive members.

Consensus pattern G-x(8,9)-G-x-[STA]-H-[LIVMFY]-[LIVMC]-[DERN]-[HRKL]-[LMFAT]-x-[LFSTH]-x-[GSTAN]-[GST] [The two H are zinc ligands] [E is the active site residue] Sequences known to belong to this class detected by the pattern ALL active members as well as all MPP alpha subunits and core II subunits. Does not detect inactive core I subunits.

Note: these proteins belong to family M16 in the classification of peptidases [5].

□

[1] Rawlings N.D., Barrett A.J. Biochem. J. 275:389-391(1991).

□

[2] Braun H.-P., Schmitz U.K. Trends Biochem. Sci. 20:171-175(1995).

[3] Becker A.B., Roth R.A. Proc. Natl. Acad. Sci. U.S.A. 89:3835-3839(1992).

[4] Fujita A., Oka C., Arikawa Y., Katagai T., Tonouchi A., Kuhara S., Misumi Y. Nature 372:567-570(1994).

[5] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

310. Involucrin repeat

Eckert RL, Yaffe MB, Crish JF, Murthy S, Rorke EA, Welter JF, J Invest Dermatol 1993;100:613-617.

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311. Isochorismatase family. This family are hydrolase enzymes.

Romao MJ, Turk D, Gomis-Ruth FX, Huber R, Schumacher G, Mollering H, Russmann L, J Mol Biol 1992;226:1111-1130.

312. Inositol monophosphatase family signatures (inositol_P)

It has been shown [1] that several proteins share two sequence motifs. Two of these proteins are enzymes of the inositol phosphate second messenger signaling pathway: - Vertebrate and plants inositol monophosphatase (EC <u>3.1.3.25</u>). - Vertebrate inositol polyphosphate 1-phosphatase (EC <u>3.1.3.57</u>).The function of the other proteins is not yet clear: - Bacterial protein cysQ. CysQ could help to control the pool of PAPS (3'-phosphoadenoside 5'-phosphosulfate), or be useful in sulfite synthesis. - Escherichia coli protein suhB. Mutations in suhB results in the enhanced synthesis of heat shock sigma factor (htpR). - Neurospora crassa protein Qa-X. Probably involved in quinate metabolism. - Emericella nidulans protein qutG. Probably involved in quinate metabolism. - Yeast protein HAL2/MET22 [2] involved in salt tolerance as well as methionine biosynthesis. - Yeast hypothetical hypothetical protein YHR046c. - Caenorhabditis elegans hypothetical protein F13G3.5. - A Rhizobium leguminosarum hypothetical protein encoded upstream of the pss gene for exopolysaccharide synthesis. - Methanococcus jannaschii hypothetical protein MJ0109.It is suggested [1] that these proteins may act by enhancing the synthesis or degradation of phosphorylated messenger molecules. From the X-ray structure of human inositol monophosphatase [3], it seems that some of the conserved residues are involved in binding a metal ion and/or the phosphate group of the substrate.

Consensus pattern: [FWV]-x(0,1)-[LIVM]-D-P-[LIVM]-D-[SG]-[ST]-x(2)-[FY]-x-[HKRNSTY] [The first D and the T bind a metal ion]-
Consensus pattern: [WV]-D-x-[AC]-[GSA]-[GSAPV]-x-[LIVACP]-[LIV]-[LIVAC]-x(3)-[GH]-[GA]-

[1] Neuwald A.F., York J.D., Majerus P.W. FEBS Lett. 294:16-18(1991).
[2] Glaeser H.-U., Thomas D., Gaxiola R., Montrichard F., Surdin-Kerjan Y., Serrano R. EMBO J. 12:3105-3110(1993).
[3] Bone R., Springer J.P., Atack J.R. Proc. Natl. Acad. Sci. U.S.A. 89:10031-10035(1992).

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313. Ion transport protein

This family contains Sodium, Potassium, Calcium ion channel This family is 6 transmembrane helices in which the last two helices flank a loop which determines ion selectivity. In some sub-families (e.g. Na channels) the domain is repeated four times, whereas in others (e.g. K channels) the protein forms as a tetramer in the membrane. A bacterial structure of the protein is known for the last two helices but is not the Pfam family due to it lacking the first four helices

314. Isocitrate and isopropylmalate dehydrogenases signature (isodh)

Isocitrate dehydrogenase (IDH) [1,2] is an important enzyme of carbohydrate metabolism which catalyzes the oxidative decarboxylation of isocitrate into alpha-ketoglutarate. IDH is either dependent on NAD+ (EC 1.1.1.41) or on NADP+(EC 1.1.1.42). In eukaryotes there are at least three isozymes of IDH: two are located in the mitochondrial matrix (one NAD+-dependent, the other NADP+-dependent), while the third one (also NADP+-dependent) is cytoplasmic. In Escherichia coli the activity of a NADP+-dependent form of the enzyme is controlled by the phosphorylation of a serine residue; the phosphorylated form of IDH is completely inactivated. 3-isopropylmalate dehydrogenase (EC 1.1.1.85) (IMDH) [3,4] catalyzes the third step in the biosynthesis of leucine in bacteria and fungi, the oxidative decarboxylation of 3-isopropylmalate into 2-oxo-4-methylvalerate. Tartrate dehydrogenase (EC 1.1.1.93) [5] catalyzes the reduction of tartrate to oxaloglycolate. These enzymes are evolutionary related [1,3,4,5]. The best conserved region of these enzymes is a glycine-rich stretch of residues located in the C-terminal section. This region was used as a signature pattern.

Consensus pattern: [NS]-[LIMYT]-[FYDN]-G-[DNT]-[IMVY]-x-[STGDN]-[DN]-x(2)-[SGAP]-x(3,4)-G-[STG]-[LIVMPA]-G-[LIVMF]-

[1] Hurley J.H., Thorsness P.E., Ramalingam V., Helmers N.H., Koshland D.E. Jr., Stroud R.M. Proc. Natl. Acad. Sci. U.S.A. 86:8635-8639(1989).
[2] Cupp J.R., McAlister-Henn L. J. Biol. Chem. 266:22199-22205(1991).
[3] Imada K., Sato M., Tanaka N., Katsube Y., Matsuura Y., Oshima T. J. Mol. Biol. 222:725-738(1991).
[4] Zhang T., Koshland D.E. Jr. Protein Sci. 4:84-92(1995).
[5] Tipton P.A., Beecher B.S. Arch. Biochem. Biophys. 313:15-21(1994).

315. Jacalin-like lectin domain.

Proteins containing this domain are lectins. It is found in 1 to 6 copies in these proteins. The domain is also found in the animal prostatic spermine-binding protein (Swiss:P15501).

[1] Sankaranarayanan R, Sekar K, Banerjee R, Sharma V, Surolia A, Vijayan M; Nat Struct Biol 1996;3:596-603.

316. KH domain

KH motifs probably bind RNA directly. Auto antibodies to Nova, a KH domain protein, cause paraneoplastic opsoclonus ataxia.

[1] Burd CG, Dreyfuss G, Science 1994;265:615-621.

[2] Musco G, Stier G, Joseph C, Castiglione Morelli MA, Nilges M, Gibson TJ, Pastore A, Cell 1996;85:237-245.

317. Kelch motif

The kelch motif was initially discovered in Kelch (Swiss:Q04652). In this protein there are six copies of the motif. It has been shown that Swiss:Q04652 is related to Galactose Oxidase [1] for which a structure has been solved [2]. The kelch motif forms a beta sheet. Several of these sheets associate to form a beta propeller structure as found in neur,

[1] Bork P, Doolittle RF, J Mol Biol 1994;236:1277-1282. [2] Ito N, Phillips SE, Stevens C, Ogel ZB, McPherson MJ, Keen, JN, Yadav KD, Knowles PF, Nature 1991;350:87-90.

09553569.091601

318. Soybean trypsin inhibitor (Kunitz) protease inhibitors family signature

The soybean trypsin inhibitor (Kunitz) family [1] is one of the numerous families of proteinase inhibitors. It comprise plant proteins which have inhibitory activity against serine proteinases from the trypsin and subtilisin families, thiol proteinases and aspartic proteinases as well as some proteins that are probably involved in seed storage. This family is currently known to group the following proteins: - Trypsin inhibitors A, B, C, KTI1, and KTI2 from soybean. - Trypsin inhibitor DE3 from coral beans (Erythrina sp.). - Trypsin inhibitor DE5 from sandal bead tree. - Trypsin inhibitors 1A (WTI-1A), 1B (WTI-1B), and 2 (WTI-2) from goa bean. - Trypsin inhibitor from Acacia confusa. - Trypsin inhibitor from silk tree. - Chymotrypsin inhibitor 3 (WCI-3) from goa bean. - Cathepsin D inhibitors PDI and NDI from potato [2], which inhibit both cathepsin D (aspartic proteinase) and trypsin. - Alpha-amylase/subtilisin inhibitors from barley and wheat. - Albumin-1 (WBA-1) from goa bean seeds [3]. - Miraculin from Richadella dulcifica [4], a sweet taste protein. - Sporamin from sweet potato [5], the major tuberous root protein. - Thiol proteinase inhibitor PCPI 8.3 (P340) from potato tuber [6]. - Wound responsive protein gwin3 from poplar tree [7]. - 21 Kd seed protein from cocoa [8].All these proteins contain from 170 to 200 amino acid residues and one or twointrachain disulfide bonds. The best conserved region is found in their N-terminal section and is used as a signature pattern

Consensus pattern: [LIVM]-x-D-x-[EDNTY]-[DG]-[RKHDENQ]-x-[LIVM]-x(5)-Y-x-[LIVM] -

[1] Laskowski M., Kato I. Annu. Rev. Biochem. 49:593-626(1980).
[2] Ritonja A., Krizaj I., Mesko P., Kopitar M., Lucovnik P., Strukelj B., Pungercar J., Buttle D.J., Barrett A.J., Turk V. FEBS Lett. 267:13-15(1990).
[3] Kortt A.A., Strike P.M., de Jersey J. Eur. J. Biochem. 181:403-408(1989).
[4] Theerasilp S., Hitotsuya H., Nakajo S., Nakaja K., Nakamura Y., Kurihara Y. J. Biol. Chem. 264:6655-6659(1989).
[5] Hattori T., Yoshida N., Nakamura K. Plant Mol. Biol. 13:563-572(1989).
[6] Krizaj I., Drobnic-Kosorok M., Brzin J., Jerala R., Turk V. FEBS Lett. 333:15-20(1993).
[7] Bradshaw H.D., Hollick J.B., Parsons T.J., Clarke H.R.G., Gordon M.P. Plant Mol. Biol. 14:51-59(1989).
[8] Tai H., McHenry L., Fritz P.J., Furtek D.B. Plant Mol. Biol. 16:913-915(1991).

319. Beta-ketoacyl synthases active site

Beta-ketoacyl-ACP synthase (KAS) [1] is the enzyme that catalyzes the condensation of malonyl-ACP with the growing fatty acid chain. It is found as a component of the following enzymatic systems: - Fatty acid synthetase (FAS), which catalyzes the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH. Bacterial and plant chloroplast FAS are composed of eight separate subunits which correspond to different enzymatic activities; beta-ketoacyl synthase is one of these polypeptides. Fungal FAS consists of two multifunctional proteins, FAS1 and FAS2; the beta-ketoacyl synthase domain is located in the C-terminal section of FAS2. Vertebrate FAS consists of a single multifunctional chain; the beta-ketoacyl synthase domain is located in the N-terminal section [2]. - The multifunctional 6-methysalicylic acid synthase (MSAS) from Penicillium patulum [3]. This is a multifunctional enzyme involved in the biosynthesis of a polyketide antibiotic and which has a KAS domain in its N-terminal section. - Polyketide antibiotic synthase enzyme systems. Polyketides are secondary metabolites produced by microorganisms and plants from simple fatty acids. KAS is one of the components involved in the biosynthesis of the Streptomyces polyketide antibiotics granatacin [4], tetracenomycin C [5] and erythromycin. - Emericella nidulans multifunctional protein Wa. Wa is involved in the biosynthesis of conidial green pigment. Wa is protein of 216 Kd that contains a KAS domain. - Rhizobium nodulation protein nodE, which probably acts as a beta-ketoacyl synthase in the synthesis of the nodulation Nod factor fatty acyl chain. - Yeast mitochondrial protein CEM1. The condensation reaction is a two step process: the acyl component of an activated acyl primer is transferred to a cysteine residue of the enzyme and is then condensed with an activated malonyl donor with the concomitant release of carbon dioxide. The sequence around the active site cysteine is well conserved and can be used as a signature pattern.

Consensus pattern: G-x(4)-[LIVMFAP]-x(2)-[AGC]-C-[STA](2)-[STAG]-x(3)-[LIVMF] [C is the active site residue]

[1] Kauppinen S., Siggaard-Andersen M., von Wettstein-Knowles P. Carlsberg Res. Commun. 53:357-370(1988).
[2] Witkowski A., Rangan V.S., Randhawa Z.I., Amy C.M., Smith S. Eur. J. Biochem. 198:571-579(1991).
[3] Beck J., Ripka S., Siegner A., Schiltz E., Schweizer E. Eur. J. Biochem. 192:487-498(1990).
[4] Bibb M.J., Biro S., Motamedi H., Collins J.F., Hutchinson C.R. EMBO J. 8:2727-2736(1989).
[5] Sherman D.H., Malpartida F., Bibb M.J., Kieser H.M., Bibb M.J., Hopwood D.A. EMBO J. 8:2717-2725(1989).

320. Kinesin motor domain signature and profile

Kinesin [1,2,3] is a microtubule-associated force-producing protein that mayplay a role in organelle transport. Kinesin is an oligomeric complex composedof two heavy chains and two light chains. The kinesin motor activity isdirected toward the microtubule's plus end.The heavy chain is composed of three structural domains: a large globular N-terminal domain which is responsible for the motor activity of kinesin (it isknown to hydrolyze ATP, to bind and move on microtubules), a central alpha-helical coiled coil domain that mediates the heavy chain dimerization; and asmall globular C-terminal domain which interacts with other proteins (such asthe kinesin light chains), vesicles and membranous organelles.A number of proteins have been recently found that contain a domain similarto that of the kinesin 'motor' domain [1,4,E1]: - Drosophila claret segregational protein (ncd). Ncd is required for normal chromosomal segregation in meiosis, in females, and in early mitotic divisions of the embryo. The ncd motor activity is directed toward the microtubule's minus end. - Drosophila kinesin-like protein (nod). Nod is required for the distributive chromosome segregation of nonexchange chromosomes during meiosis. - Human CENP-E [4]. CENP-E is a protein that associates with kinetochores during chromosome congression, relocates to the spindle midzone at anaphase, and is quantitatively discarded at the end of the cell division. CENP-E is probably an important motor molecule in chromosome movement and/ or spindle elongation. - Human mitotic kinesin-like protein-1 (MKLP-1), a motor protein whose activity is directed toward the microtubule's plus end. - Yeast KAR3 protein, which is essential for yeast nuclear fusion during mating. KAR3 may mediate microtubule sliding during nuclear fusion and possibly mitosis. - Yeast CIN8 and KIP1 proteins which are required for the assembly of the mitotic spindle. Both proteins seem to interact with spindle microtubules to produce an outwardly directed force acting upon the poles. - Fission yeast cut7 protein, which is essential for spindle body duplication during mitotic division. - Emericella nidulans bimC, which plays an important role in nuclear division. - Emericella nidulans klpA. - Caenorhabditis elegans unc-104, which may be required for the transport of substances needed for neuronal cell differentiation. - Caenorhabditis elegans osm-3. - Xenopus Eg5, which may be involved in mitosis. - Arabidopsis thaliana KatA, KatB and katC. - Chlamydomonas reinhardtii FLA10/KHP1 and KLP1. Both proteins seem to play a role in the rotation or twisting of the microtubules of the flagella. - Caenorhabditis elegans hypothetical protein T09A5.2.The kinesin motor domain is located in the N-terminal part of most of theabove proteins, with the exception of KAR3, klpA, and ncd where it is locatedin the C-terminal section.The kinesin motor domain contains about 330 amino acids. An ATP-binding motifof type A is found near position 80 to 90, the C-terminal half of the domainis involved in microtubule-binding. The signature pattern for that domain isderived from a conserved decapeptide inside the microtubule-binding part.

Consensus pattern: [GSA]-[KRHPSTQVM]-[LIVMF]-x-[LIVMF]-[IVC]-D-L-[AH]-G-[SAN]-E

[1] Bloom G.S., Endow S.A. Protein Prof. 2:1109-1171(1995).

[2] Vallee R.B., Shpetner H.S. Annu. Rev. Biochem. 59:909-932(1990).

[3] Brady S.T. Trends Cell Biol. 5:159-164(1995).

[4] Endow S.A. Trends Biochem. Sci. 16:221-225(1991).[E1]

321. Ribosomal protein L15 signature

Ribosomal protein L15 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L15 is known to bind the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L15. - Plant chloroplast L15 (nuclear-encoded). - Archaebacterial L15. - Vertebrate L27a. - Tetrahymena thermophila L29. - Fungi L27a (L29, CRP-1, CYH2).L15 is a protein of 144 to 154 amino-acid residues. As a signature pattern, a conserved region was selected in the C-terminal section of these proteins.

Consensus pattern: K-[LIVM](2)-[GASL]-x-[GT]-x-[LIVMA]-x(2,5)-[LIVM]-x- [LIVMF]-x(3,4)-[LIVMFCA]-[ST]-x(2)-A-x(3)-[LIVM]-x(3)-G

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

322. LBP / BPI / CETP family signature

The following mammalian lipid-binding serum glycoproteins belong to the same family [1,2,3]: - Lipopolysaccharide-binding protein (LBP). LBP binds to the lipid A moiety of bacterial lipopolysaccharides (LPS), a glycolipid present in the outer membrane of all Gram-negative bacteria. The LBP/LPS complex seems to interact with the CD14 receptor and may be responsible for the secretion of alpha-TNF. - Bactericidal permeability-increasing protein (BPI). Like LBP, BPI binds LPS and has a cytotoxic activity on Gram-negative bacteria. - Cholesteryl ester transfer protein (CETP). CETP is involved in the transfer of insoluble cholesteryl esters in reverse cholesterol transport. - Phospholipid transfer protein (PLTP). May play a key role in extracellular phospholipid transport and modulation of HDL particles. These proteins are structurally related and share many regions of sequencesimilarities. As a signature pattern one of these regions was selected, which is located in the N-terminal section of these proteins; a region which could be involved in the binding to the lipids [2].

09554526.020700

Consensus pattern: [PA]-[GA]-[LIVMC]-x(2)-R-[IV]-[ST]-x(3)-L-x(5)-[EQ]-x(4)- [LIVM]-[EQK]-x(8)-P

[1] Schumann R.R., Leong S.R., Flaggs G.W., Gray P.W., Wright S.D., Mathison J.C., Tobias P.S., Ulevitch R.J. Science 249:1429-1431(1990).

[2] Gray P.W., Flaggs G., Leong S.R., Gumina R.J., Weiss J., Ooi C.E., Elsbach P. J. Biol. Chem. 264:9505-9509(1989).

[3] Day J.R., Albers J.J., Lofton-Day C.E., Gilbert T.L., Ching A.F.T., Grant F.J., O'Hara P.J., Marcovina S.M., Adolphson J.L. J. Biol. Chem. 269:9388-9391(1994).

09554359.072601

323. LIM domain signature and profile

Recently [1,2] a number of proteins have been found to contain a conserved cysteine-rich domain of about 60 amino-acid residues. These proteins are: - Caenorhabditis elegans mec-3; a protein required for the differentiation of the set of six touch receptor neurons in this nematode. - Caenorhabditis elegans lin-11; a protein required for the asymmetric division of vulval blast cells. - Vertebrate insulin gene enhancer binding protein isl-1. Isl-1 binds to one of the two cis-acting protein-binding domains of the insulin gene. - Vertebrate homeobox proteins lim-1, lim-2 (lim-5) and lim3. - Vertebrate lmx-1, which acts as a transcriptional activator by binding to the FLAT element; a beta-cell-specific transcriptional enhancer found in the insulin gene. - Mammalian LH-2, a transcriptional regulatory protein involved in the control of cell differentiation in developing lymphoid and neural cell types. - Drosophila protein apterous, required for the normal development of the wing and halter imaginal discs. - Vertebrate protein kinases LIMK-1 and LIMK-2. - Mammalian rhombotins. Rhombotin 1 (RBTN1 or TTG-1) and rhombotin-2 (RBTN2 or TTG-2) are proteins of about 160 amino acids whose genes are disrupted by chromosomal translocations in T-cell leukemia. - Mammalian and avian cysteine-rich protein (CRP), a 192 amino-acid protein of unknown function. Seems to interact with zyxin. - Mammalian cysteine-rich intestinal protein (CRIP), a small protein which seems to have a role in zinc absorption and may function as an intracellular zinc transport protein. - Vertebrate paxillin, a cytoskeletal focal adhesion protein. - Mouse testin. Mouse testin should not be confused with rat testin which is a thiol protease homolog. - Sunflower pollen specific protein SF3. - Chicken zyxin. Zyxin is a low-abundance adhesion plaque protein which has been shown to interact with CRP. - Yeast protein LRG1 which is involved in sporulation [4]. - Yeast rho-type GTPase activating protein RGA1/DBM1. - Caenorhabditis elegans homeobox protein ceh-14. - Caenorhabditis elegans homeobox protein unc-97. - Yeast hypothetical protein YKR090w. - Caenorhabditis elegans hypothetical proteins C28H8.6.These proteins generally have two tandem copies of a domain, called LIM (forLin-11 Isl-1 Mec-3) in their N-terminal section. Zyxin and paxillin areexceptions in that they contains respectively three and four LIM domains attheir C-terminal extremity. In apterous, isl-1, LH-2, lin-11, lim-1 to lim-3,lmx-1 and ceh-14 and mec-3 there is a homeobox domain some 50 to 95 amino acids after theLIM domains.In the LIM domain, there are seven conserved cysteine residues and ahistidine. The arrangement followed by these conserved residues is C-x(2)-C-x(16,23)-H-x(2)-[CH]-x(2)-C-x(2)-C-x(16,21)-C-x(2,3)-[CHD]. The LIM domainbinds two zinc ions [5]. LIM does not bind DNA, rather it seems to act asinterface for protein-protein interaction. A pattern was developed that spans the first half of the LIM domain.

09553960.091500

Consensus pattern: C-x(2)-C-x(15,21)-[FYWH]-H-x(2)-[CH]-x(2)-C-x(2)-C-x(3)- [LIVMF] [The 5 C's and the H bind zinc]

[1] Freyd G., Kim S.K., Horvitz H.R. Nature 344:876-879(1990).

[2] Baltz R., Evrard J.-L., Domon C., Steinmetz A. Plant Cell 4:1465-1466(1992).

[3] Sanchez-Garcia I., Rabbitts T.H. Trends Genet. 10:315-320(1994).

[4] Mueller A., Xu G., Wells R., Hollenberg C.P., Piepersberg W. Nucleic Acids Res. 22:3151-3154(1994).

[5] Michelsen J.W., Schmeichel K.L., Beckerle M.C., Winge D.R. Proc. Natl. Acad. Sci. U.S.A. 90:4404-4408(1993).

324. (LRR) Leucine Rich Repeat

CAUTION: This Pfam may not find all Leucine Rich Repeats in a protein. Leucine Rich Repeats are short sequence motifs present in a number of proteins with diverse functions and cellular locations. These repeats are usually involved in protein-protein interactions. Each Leucine Rich Repeat is composed of a beta-alpha unit. These units form elongated non-globular structures. Leucine Rich Repeats are often flanked by cysteine rich domains.

Number of members: 3017

[1] The leucine-rich repeat: a versatile binding motif. Kobe B, Deisenhofer J; Trends Biochem Sci 1994;19:415-421. [2] Crystal structure of porcine ribonuclease inhibitor, a protein with leucine-rich repeats. Kobe B, Deisenhofer J; Nature 1993;366:751-756.

325. Plant lipid transfer protein family signature (LTP)

Plant cells contain proteins, called lipid transfer proteins (LTP) [1,2,3], which are able to facilitate the transfer of phospholipids and other lipidsacross membranes. These proteins, whose subcellular location is not yet known, could play a major role in membrane biogenesis by conveying phospholipids such as waxes or cutin from their site of biosynthesis to membranes unable to form these lipids. Plant LTP's are proteins of about 9 Kd (90 amino acids) which contain eight conserved cysteine residues all involved in disulfide bridges, as shown in the following schematic representation.

```
+----------------------+ | +------+ | | | | | ***************
xCxxxxCxxxxxxCCxxxxxxxxCxCxxxxxxxxxxxCxxxxxxCxx | | | | +--------|------------+ | +---
-----------------+
```

'C': conserved cysteine involved in a disulfide bond.
'*': position of the pattern.

Consensus pattern: [LIVM]-[PA]-x(2)-C-x-[LIVM]-x-[LIVM]-x-[LIVMFY]-x-[LIVM]-[ST]-x(3)-[DN]-C-x(2)-[LIVM] [The two C's are involved in disulfide bonds]

[1] Wirtz K.W.A. Annu. Rev. Biochem. 60:73-99(1991).
[2] Arondel V., Kader J.C. Experientia 46:579-585(1990).
[3] Ohlrogge J.B., Browse J., Somerville C.R. Biochim. Biophys. Acta 1082:1-26(1991).

326. (LAMP) Lysosome-associated membrane glycoproteins signatures

Lysosome-associated membrane glycoproteins (lamp) [1] are integral membrane proteins, specific to lysosomes, and whose exact biological function is not yet clear. Structurally, the lamp proteins consist of two internally homologous lysosome-luminal domains separated by a proline-rich hinge region; at the C-terminal extremity there is a transmembrane region followed by a very short cytoplasmic tail. In each of the duplicated domains, there are two conserved disulfide bonds. This structure is schematically represented in the figure below. +-----+ +-----+ +-----+ +-----+ | | | | | | | | xCxxxxxCxxxxxxxxxxxxCxxxxxCxxxxxxxxxxCxxxxxCxxxxxxxxxxxxCxxxxxCxxxxxxxxx <------------------------><Hinge><------------------------><TM><C> In mammals, there are two closely related types of lamp: lamp-1 and lamp-2. In chicken lamp-1 is known as LEP100.The macrophage protein CD68 (or macrosialin) [2] is a heavily glycosylatedintegral membrane protein whose structure consists of a mucin-like domain followed by a proline-rich hinge; a single lamp-like domain; a transmembrane region and a short cytoplasmic tail. Two signature patterns for this family of proteins were developed. The first oneis centered on the first conserved cysteine of the duplicated domains. The second corresponds to a region that includes the extremity of the second domain, the totality of the transmembrane region and the cytoplasmic tail.

Consensus pattern: [STA]-C-[LIVM]-[LIVMFYW]-A-x-[LIVMFYW]-x(3)-[LIVMFYW]-x(3)-Y [C is involved in a disulfide bond] –
Consensus pattern: C-x(2)-D-x(3,4)-[LIVM](2)-P-[LIVM]-x-[LIVM]-G-x(2)-[LIVM]- x-G-[LIVM](2)-x-[LIVM](4)-A-[FY]-x-[LIVM]-x(2)-[KR]-[RH]- x(1,2)-[STAG](2)-Y-[EQ] [C is involved in a disulfide bond]

[1] Fukuda M. J. Biol. Chem. 266:21327-21330(1991).
[2] Holness C.L., da Silva R.P., Fawcett J., Gordon S., Simmons D.L. J. Biol. Chem. 268:9661-9666(1993).

327. Lipolytic enzymes "G-D-S-L" family, serine active site

Recently [1], a family of lipolytic enzymes has been characterized. This family currently consist of the following proteins:

- Aeromonas hydrophila lipase/phosphatidylcholine-sterol acyltransferase.
- Xenorhabdus luminescens lipase 1.
- Vibrio mimicus arylesterase.
- Escherichia coli acyl-coA thioesterase I (gene tesA).
- Vibrio parahaemolyticus thermolabile hemolysin/atypical phospholipase.

- Rabbit phospholipase AdRab-B, an intestinal brush border protein with esterase and phospholipase A/lysophospholipase activity that could be involved in the uptake of dietary lipids. AdRab-B contains four repeats of about 320 amino acids.
- Arabidopsis thaliana and Brassic napus anther-specific proline-rich protein APG.
- A Pseudomonas putida hypothetical protein in trpE-trpG intergenic region. A serine has been identified a part of the active site in the Aeromonas, Vibrio mimicus and Escherichia coli enzymes. It is located in a conserved sequence motif that can be used as a signature pattern for these proteins.

-Consensus pattern: [LIVMFYAG](4)-G-D-S-[LIVM]-x(1,2)-[TAG]-G
[S is the active site residue]

09555365.040700

328. (Lipoprotein 4) Prokaryotic membrane lipoprotein lipid attachment site

In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signalpeptidase II). The peptidase recognizes a conserved sequence and cuts upstreamof a cysteine residue to which a glyceride-fatty acid lipid is attached [1].Some of the proteins known to undergo such processing currently include (forrecent listings see [1,2,3]): - Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp). - Escherichia coli lipoprotein-28 (gene nlpA). - Escherichia coli lipoprotein-34 (gene nlpB). - Escherichia coli lipoprotein nlpC. - Escherichia coli lipoprotein nlpD. - Escherichia coli osmotically inducible lipoprotein B (gene osmB). - Escherichia coli osmotically inducible lipoprotein E (gene osmE). - Escherichia coli peptidoglycan-associated lipoprotein (gene pal). - Escherichia coli rare lipoproteins A and B (genes rplA and rplB). - Escherichia coli copper homeostasis protein cutF (or nlpE). - Escherichia coli plasmids traT proteins. - Escherichia coli Col plasmids lysis proteins. - A number of Bacillus beta-lactamases. - Bacillus subtilis periplasmic oligopeptide-binding protein (gene oppA). - Borrelia burgdorferi outer surface proteins A and B (genes ospA and ospB). - Borrelia hermsii variable major protein 21 (gene vmp21) and 7 (gene vmp7). - Chlamydia trachomatis outer membrane protein 3 (gene omp3). - Fibrobacter succinogenes endoglucanase cel-3. - Haemophilus influenzae proteins Pal and Pcp. - Klebsiella pullulunase (gene pulA). - Klebsiella pullulunase secretion protein pulS. - Mycoplasma hyorhinis protein p37. - Mycoplasma hyorhinis variant surface antigens A, B, and C (genes vlpABC). -

Neisseria outer membrane protein H.8. - Pseudomonas aeruginosa lipopeptide (gene lppL). - Pseudomonas solanacearum endoglucanase egl. - Rhodopseudomonas viridis reaction center cytochrome subunit (gene cytC). - Rickettsia 17 Kd antigen. - Shigella flexneri invasion plasmid proteins mxiJ and mxiM. - Streptococcus pneumoniae oligopeptide transport protein A (gene amiA). - Treponema pallidium 34 Kd antigen. - Treponema pallidium membrane protein A (gene tmpA). - Vibrio harveyi chitobiase (gene chb). - Yersinia virulence plasmid protein yscJ. - Halocyanin from Natrobacterium pharaon is [4], a membrane associated copper- binding protein. This is the first archaebacterial protein known to be modified in such a fashion).From the precursor sequences of all these proteins, a consensus pattern and a set of rules to identify this type of post-translational modification was derived.

09555326.091500

Consensus pattern: {DERK}(6)-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence.

[1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).
[2] Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).
[3] von Heijne G. Protein Eng. 2:531-534(1989).
[4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

329. (Lopoprotein 5) Prokaryotic membrane lipoprotein lipid attachment site. In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which a glyceride-fatty acid lipid is attached [1].Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]): - Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp). - Escherichia coli lipoprotein-28 (gene nlpA). - Escherichia coli lipoprotein-34 (gene nlpB). - Escherichia coli lipoprotein nlpC. - Escherichia coli lipoprotein nlpD. - Escherichia coli osmotically inducible lipoprotein B (gene osmB). - Escherichia coli osmotically inducible lipoprotein E (gene osmE). - Escherichia coli peptidoglycan-associated

lipoprotein (gene pal). - Escherichia coli rare lipoproteins A and B (genes rplA and rplB). - Escherichia coli copper homeostasis protein cutF (or nlpE). - Escherichia coli plasmids traT proteins. - Escherichia coli Col plasmids lysis proteins. - A number of Bacillus beta-lactamases. - Bacillus subtilis periplasmic oligopeptide-binding protein (gene oppA). - Borrelia burgdorferi outer surface proteins A and B (genes ospA and ospB). - Borrelia hermsii variable major protein 21 (gene vmp21) and 7 (gene vmp7). - Chlamydia trachomatis outer membrane protein 3 (gene omp3). - Fibrobacter succinogenes endoglucanase cel-3. - Haemophilus influenzae proteins Pal and Pcp. - Klebsiella pullulunase (gene pulA). - Klebsiella pullulunase secretion protein pulS. - Mycoplasma hyorhinis protein p37. - Mycoplasma hyorhinis variant surface antigens A, B, and C (genes vlp ABC). - Neisseria outer membrane protein H.8. - Pseudomonas aeruginosa lipopeptide (gene lppL). - Pseudomonas solanacearum endoglucanase egl. - Rhodopseudomonas viridis reaction center cytochrome subunit (gene cytC). - Rickettsia 17 Kd antigen. - Shigella flexneri invasion plasmid proteins mxiJ and mxiM. - Streptococcus pneumoniae oligopeptide transport protein A (gene amiA). - Treponema pallidium 34 Kd antigen. - Treponema pallidium membrane protein A (gene tmpA). - Vibrio harveyi chitobiase (gene chb). - Yersinia virulence plasmid protein yscJ. - Halocyanin from Natrobacterium pharaonis [4], a membrane associated copper- binding protein. This is the first archaebacterial protein known to be modified in such a fashion).From the precursor sequences of all these proteins, a consensus pattern and a set of rules to identify this type of post-translational modification have been developed.

Consensus pattern: {DERK}(6)-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence.

[1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).[2] Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).[3] von Heijne G. Protein Eng. 2:531-534(1989).[4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

330. (Lum binding) Riboflavin synthase alpha chain family Lum-binding site signature

The following proteins have been shown [1,2] to be structurally and evolutionary related: - Riboflavin synthase alpha chain (RS-alpha) (gene ribC in Escherichia coli, ribB in Bacillus subtilis and Photobacterium leiognathi, RIB5 in yeast). This enzyme synthesizes riboflavin from two moles of 6,7- dimethyl-8-(1'-D-ribityl)lumazine (Lum), a pteridine-derivative. - Photobacterium phosphoreum lumazine protein (LumP) (gene luxL). LumP is a protein that modulates the color of the bioluminescence emission of bacterial luciferase. In the presence of LumP, light emission is shifted to higher energy values (shorter wavelength). LumP binds non-covalently to 6,7-dimethyl-8-(1'-D-ribityl) lumazine. - Vibrio fischeri yellow fluorescent protein (YFP) (gene luxY). Like LumP, YFP modulates light emission but towards a longer wavelength. YFP binds non-covalently to FMN. These proteins seem to have evolved from the duplication of a domain of about100 residues. In its C-terminal section, this domain contains a conserved motif [KR]-V-N-[LI]-E which has been proposed to be the binding site for Lum.RS-alpha which binds two molecules of Lum has two perfect copies of this motif, while LumP which binds one molecule of Lum, has a Glu instead of Lys/Arg in the first position of the second copy of the motif. Similarily, YFP, which binds to one molecule of FMN, also seems to have a potentially dysfunctional binding site by substitution of Gly for Glu in the last positionof the first copy of the motif. Our signature pattern includes the Lum-binding motif.

Consensus pattern: [LIVMF]-x(5)-G-[STADNQ]-[KREQIYW]-V-N-[LIVM]-E

[1] O'Kane D.J., Woodward B., Lee J., Prasher D.C. Proc. Natl. Acad. Sci. U.S.A. 88:1100-1104(1991).
[2] O'Kane D.J., Prasher D.C. Mol. Microbiol. 6:443-449(1992).

331. Lysyl oxidase putative copper-binding region signature

Lysyl oxidase (LOX) [1] is an extracellular copper-dependent enzyme that catalyzes the oxidative deamination of peptidyl lysine residues in precursors of various collagens and elastins. The deaminated lysines are then able to form aldehyde cross-links.LOX binds a single copper atom which seems to reside within an octahedral coordination complex which includes at least three histidine ligands. Fourhistidine residues are clustered in a central region of the enzyme. This region is thought to be involved in cooper-binding and is called the 'copper-talon' [1]. This region was used as a signature pattern.

Consensus pattern: W-E-W-H-S-C-H-Q-H-Y-H

[1] Krebs C.J., Krawetz S.A. Biochim. Biophys. Acta 1202:7-12(1993).

332. Metallo-beta-lactamase superfamily (lactamase_B)

[1] : Neuwald AF, Liu JS, Lipman DJ, Lawrence CE, Nucleic Acids Res 1997;25:1665-1677. [2] Carfi A, Pares S, Duee E, Galleni M, Duez C, Frere JM, Dideberg O, EMBO J 1995;14:4914-4921.

333. L-lactate dehydrogenase active site (ldh1)

L-lactate dehydrogenase (EC 1.1.1.27) (LDH) [1] catalyzes the reversible NAD-dependent interconversion of pyruvate to L-lactate. In vertebrate muscles and in lactic acid bacteria it represents the final step in anaerobic glycolysis. This tetrameric enzyme is present in prokaryotic and eukaryotic organisms. Invertebrates there are three isozymes of LDH: the M form (LDH-A), found predominantly in muscle tissues; the H form (LDH-B), found in heart muscle and the X form (LDH-C), found only in the spermatozoa of mammals and birds. In birds and crocodilian eye lenses, LDH-B serves as a structural protein and is known as epsilon-crystallin [2].L-2-hydroxyisocaproate dehydrogenase (EC 1.1.1.-) (L-hicDH) [3] catalyzes the reversible and stereospecific interconversion between 2-ketocarboxylic acids and L-2-hydroxy-carboxylic acids. L-hicDH is evolutionary related to LDH's. As a signature for LDH's a region was selected that includes a conserved histidine which is essential to the catalytic mechanism.

Consensus pattern: [LIVMA]-G-[EQ]-H-G-[DN]-[ST] [H is the active site residue] -

[1] Abad-Zapatero C., Griffith J.P., Sussman J.L., Rossmann M.G. J. Mol. Biol. 198:445-467(1987).

[2] Hendriks W., Mulders J.W.M., Bibby M.A., Slingsby C., Bloemendal H., de Jong W.W. Proc. Natl. Acad. Sci. U.S.A. 85:7114-7118(1988).

[3] Lerch H.-P., Frank R., Collins J. Gene 83:263-270(1989).

Malate dehydrogenase active site signature (ldh2)

Malate dehydrogenase (EC <u>1.1.1.37</u>) (MDH) [1,2] catalyzes the interconversion of malate to oxaloacetate utilizing the NAD/NADH cofactor system. The enzyme participates in the citric acid cycle and exists in all aerobic organisms. While prokaryotic organisms contains a single form of MDH, in eukaryotic cells there are two isozymes: one which is located in the mitochondrial matrix and the other in the cytoplasm. Fungi and plants also harbor a glyoxysomal form which functions in the glyoxylate pathway. In plants chloroplast there is an additional NADP-dependent form of MDH (EC <u>1.1.1.82</u>) which is essential for both the universal C3 photosynthesis (Calvin) cycle and the more specializedC4 cycle. As a signature pattern for this enzyme a region was chosen that includes two residues involved in the catalytic mechanism [3]: an aspartic acid which is involved in a proton relay mechanism, and an arginine which binds the substrate.

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Consensus pattern: [LIVM]-T-[TRKMN]-L-D-x(2)-R-[STA]-x(3)-[LIVMFY] [D and R are the active site residues]-

[1] McAlister-Henn L. Trends Biochem. Sci. 13:178-181(1988).

[2] Gietl C. Biochim. Biophys. Acta 1100:217-234(1992).

[3] Birktoft J.J., Rhodes G., Banaszak L.J. Biochemistry 28:6065-6081(1989).

[4] Cendrin F., Chroboczek J., Zaccai G., Eisenberg H., Mevarech M. Biochemistry 32:4308-4313(1993).

334. Legume lectins signatures

Leguminous plants synthesize sugar-binding proteins which are called legume lectins [1,2]. These lectins are generally found in the seeds. The exact function of legume lectins is not known but they may be involved in the attachment of nitrogen-fixing bacteria to legumes and in the protection against pathogens. Legume lectins bind calcium and manganese (or other transition metals). Legume lectins are synthesized as precursor proteins of about 230 to 260 amino acid residues. Some legume lectins are proteolytically processed to produce two chains: beta (which corresponds to the N-terminal) and alpha (C-terminal).The lectin concanavalin A (conA) from jack bean is exceptional in that the two chains are transposed and ligated (by formation of a new peptide bond). The N-terminus of mature conA thus corresponds to that of the alpha chain and the C-terminus to the beta chain. Two signature patterns specific to legume lectins have been developed: the first is located in the C-terminal section of the beta chain and contains a conserved aspartic acid residue important for the binding of calcium and manganese; the second one is located in the N-terminal of the alpha chain.

Consensus pattern: [LIV]-[STAG]-V-[DEQV]-[FLI]-D-[ST] [D binds manganese and calcium]-

Consensus pattern: [LIV]-x-[EDQ]-[FYWKR]-V-x-[LIVF]-G-[LF]-[ST]-

09555325.061600

[1] Sharon N., Lis H. FASEB J. 4:3198-320(1990).

[2] Lis H., Sharon N. Annu. Rev. Biochem. 55:33-37(1986).

335. CoA-ligases (ligases- CoA)

This family includes the CoA ligases Succinyl-CoA synthetase alpha: and beta chains, malate CoA ligase and ATP-citrate lyase. Some members of the family utilise ATP others use GTP.

[1] Wolodko WT, Fraser ME, James MN, Bridger WA, J Biol Chem 1994;269:10883-10890.

336. linker histone H1 and H5 family

Linker histone H1 is an essential component of chromatin structure. H1 links nucleosomes into higher order structures Histone H1 is replaced by histone H5 in some cell types.

[1] Ramakrishnan V, Finch JT, Graziano V, Lee PL, Sweet RM, Nature 1993;362:219-223.

337. Lipocalin signature (lip1)

Proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids, and lipids share limited regions of sequence homology and a common tertiary structure architecture [1 to 5]. This is an eight stranded antiparallel beta-barrel with a repeated + 1 topology enclosing a internal ligand binding site [1,3]. The name 'lipocalin' has been proposed [5] for this protein family. Proteins known to belong to this family are listed below (references are only provided for recently determined sequences). - Alpha-1-microglobulin (protein HC), which seems to bind porphyrin. - Alpha-1-acid glycoprotein (orosomucoid), which can bind a remarkable array of natural and synthetic compounds [6]. - Aphrodisin which, in hamsters, functions as an aphrodisiac pheromone. - Apolipoprotein D, which probably binds heme-related compounds. - Beta-lactoglobulin, a milk protein whose physiological function appears to bind retinol. - Complement component C8 gamma chain, which seems to bind retinol [7]. - Crustacyanin [8], a protein from lobster carapace, which binds astaxanthin, a carotenoid. - Epididymal-retinoic acid binding protein (E-RABP) [9] involved in sperm maturation. - Insectacyanin, a moth bilin-binding protein, and a related butterfly bilin- binding protein (BBP). - Late Lactation protein (LALP), a milk protein from tammar wallaby [10]. - Neutrophil gelatinase-associated lipocalin (NGAL) (p25) (SV-40 induced 24p3 protein) [11]. - Odorant-binding protein (OBP), which binds odorants. - Plasma retinol-binding proteins (PRBP). - Human pregnancy-associated endometrial alpha-2 globulin. - Probasin (PB), a rat prostatic protein. - Prostaglandin D synthase (EC 5.3.99.2) (GSH-independent PGD synthetase), a lipocalin with enzymatic activity [12]. - Purpurin, a retinal protein which binds retinol and heparin. - Quiescence specific protein p20K from chicken (embryo CH21 protein). - Rodent urinary proteins (alpha-2-microglobulin), which may bind pheromones. - VNSP 1 and 2, putative pheromone transport proteins from mouse vomeronasal organ [13]. - Von Ebner's gland protein (VEGP) [14] (also called tear lipocalin), a mammalian protein which may be involved in taste recognition. - A frog olfactory protein, which may transport odorants. - A protein found in the cerebrospinal fluid of the toad Bufo Marinus with a supposed function similar to transthyretin in transport across the blood brain barrier [15]. - Lizard's epididymal secretory protein IV (LESP IV), which could transport small hydrophobic molecules into the epididymal fluid during sperm maturation [16]. - Prokaryotic outer-membrane protein blc [17].The sequences of most members of the family, the core or kernal lipocalins, are characterized by three short conserved stretches of residues

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[3,18].Others, the outlier lipocalin group, share only one or two of these [3,18]. A signature pattern was built around the first, common to all outlier and kernallipocalins, which occurs near the start of the first beta-strand.

Consensus pattern: [DENG]-x-[DENQGSTARK]-x(0,2)-[DENQARK]-[LIVFY]-{CP}-G-{C}- W-[FYWLRH]-x-[LIVMTA]-
Note: it is suggested, on the basis of similarities of structure, function, and sequence, that this family forms an overall superfamily, called the calycins, with the avidin/streptavidin <PDOC00499> and the cytosolic fatty- acid binding proteins <PDOC00188> families [3,19]

[1] Cowan S.W., Newcomer M.E., Jones T.A. Proteins 8:44-61(1990).
[2] Igaraishi M., Nagata A., Toh H., Urade H., Hayaishi N. Proc. Natl. Acad. Sci. U.S.A. 89:5376-5380(1992).
[3] Flower D.R., North A.C.T., Attwood T.K. Protein Sci. 2:753-761(1993).
[4] Godovac-Zimmermann J. Trends Biochem. Sci. 13:64-66(1988).
[5] Pervaiz S., Brew K. FASEB J. 1:209-214(1987).
[6] Kremer J.M.H., Wilting J., Janssen L.H.M. Pharmacol. Rev. 40:1-47(1989).
[7] Haefliger J.-A., Peitsch M.C., Jenne D., Tschopp J. Mol. Immunol. 28:123-131(1991).
[8] Keen J.N., Caceres I., Eliopoulos E.E., Zagalsky P.F., Findlay J.B.C. Eur. J. Biochem. 197:407-417(1991).
[9] Newcomer M.E. Structure 1:7-18(1993).
[10] Collet C., Joseph R. Biochim. Biophys. Acta 1167:219-222(1993).
[11] Kjeldsen L., Johnsen A.H., Sengelov H., Borregaard N. J. Biol. Chem. 268:10425-10432(1993).
[12] Peitsch M.C., Boguski M.S. Trends Biochem. Sci. 16:363-363(1991).
[13] Miyawaki A., Matsushita Y.R., Ryo Y., Mikoshiba T. EMBO J. 13:5835-5842(1994).
[14] Kock K., Ahlers C., Schmale H. Eur. J. Biochem. 221:905-916(1994).
[15] Achen M.G., Harms P.J., Thomas T., Richardson S.J., Wettenhall R.E.H., Schreiber G. J. Biol. Chem. 267:23170-23174(1992).
[16] Morel L., Dufarre J.-P., Depeiges A. J. Biol. Chem. 268:10274-10281(1993).
[17] Bishop R.E., Penfold S.S., Frost L.S., Holtje J.V., Weiner J.H. J. Biol. Chem. 270:23097-23103(1995).

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[18] Flower D.R., North A.C.T., Attwood T.K. Biochem. Biophys. Res. Commun. 180:69-74(1991).

[19] Flower D.R. FEBS Lett. 333:99-102(1993).

Cytosolic fatty-acid binding proteins signature (lip2)

A number of low molecular weight proteins which bind fatty acids and other organic anions are present in the cytosol [1,2]. Most of them are structurally related and have probably diverged from a common ancestor. This structure is a ten stranded antiparallel beta-barrel, albeit with a wide discontinuity between the fourth and fifth strands, with a repeated + 1 topology enclosing an internal ligand binding site [2,7]. Proteins known to belong to this family include: - Six, tissue-specific, types of fatty acid binding proteins (FABPs) found in liver, intestine, heart, epidermal, adipocyte, brain/retina. Heart FABP is also known as mammary-derived growth inhibitor (MDGI), a protein that reversibly inhibits proliferation of mammary carcinoma cells. Epidermal FABP is also known as psoriasis-associated FABP [3]. - Insect muscle fatty acid-binding proteins. - Testis lipid binding protein (TLBP). - Cellular retinol-binding proteins I and II (CRBP). - Cellular retinoic acid-binding protein (CRABP). - Gastrotropin, an ileal protein which stimulates gastric acid and pepsinogen secretion. It seems that gastrotropin binds to bile salts and bilirubins. - Fatty acid binding proteins MFB1 and MFB2 from the midgut of the insect Manduca sexta [4].In addition to the above cytosolic proteins, this family also includes: - Myelin P2 protein, which may be a lipid transport protein in Schwann cells. P2 is associated with the lipid bilayer of myelin. - Schistosoma mansoni protein Sm14 [5] which seems to be involved in the transport of fatty acids. - Ascaris suum p18 a secreted protein that may play a role in sequestering potentially toxic fatty acids and their peroxidation products or that may be involved in the maintenance of the impermeable lipid layer of the eggshell. - Hypothetical fatty acid-binding proteins F40F4.2, F40F4.3, F40F4.4 and ZK742.5 from Caenorhabditis elegans. As a signature pattern for these proteins a segment from the N-terminal extremity was use.

Consensus pattern: [GSAIVK]-x-[FYW]-x-[LIVMF]-x(4)-[NHG]-[FY]-[DE]-x- [LIVMFY]-[LIVM]-x(2)-[LIVMAKR]-

Note: it is suggested, on the basis of similarities of structure, function, and sequence, that this family forms an overall superfamily, called the calycins, with the lipocalin <PDOC00187> and avidin/streptavidin <PDOC00499> families [6,7].

[1] Bernier I., Jolles P. Biochimie 69:1127-1152(1987).

[2] Veerkamp J.H., Peeters R.A., Maatman R.G.H.J. Biochim. Biophys. Acta 1081:1-24(1991).

[3] Siegenthaler G., Hotz R., Chatellard-Gruaz D., Didierjean L., Hellman U., Saurat J.-H. Biochem. J. 302:363-371(1994).

[4] Smith A.F., Tsuchida K., Hanneman E., Suzuki T.C., Wells M.A. J. Biol. Chem. 267:380-384(1992).

[5] Moser D., Tendler M., Griffiths G., Klinkert M.-Q. J. Biol. Chem. 266:8447-8454(1991).

[6] Flower D.R., North A.C.T, Attwood T.K. Protein Sci. 2:753-761(1993).

[7] Flower D.R. FEBS Lett. 333:99-102(1993).

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338. Lipoxygenases iron-binding region signatures

Lipoxygenases (EC 1.13.11.-) are a class of iron-containing dioxygenases which catalyzes the hydroperoxidation of lipids, containing a cis,cis-1,4-pentadiene structure. They are common in plants where they may be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding [1]. In mammals a number of lipoxygenases isozymes are involved in the metabolism of prostaglandins and leukotrienes [2]. Sequence data is available for the following lipoxygenases: - Plant lipoxygenases (EC 1.13.11.12). Plants express a variety of cytosolic isozymes as well as what seems [3] to be a chloroplast isozyme. - Mammalian arachidonate 5-lipoxygenase (EC 1.13.11.34). - Mammalian arachidonate 12-lipoxygenase (EC 1.13.11.31). - Mammalian erythroid cell-specific 15-lipoxygenase (EC 1.13.11.33).The iron atom in lipoxygenases is bound by four ligands, three of which are histidine residues [4]. Six histidines are conserved in all lipoxygenase sequences, five of them are found clustered in a stretch of 40 amino acids. This region contains two of the three zinc-ligands; the other histidines have been shown [5] to be important for the activity of lipoxygenases. As signatures for this family of enzymes two patterns in the region of the histidine cluster were selected. The first pattern contains the first three conserved histidines and the second pattern includes the fourth and the fifth.

Consensus pattern: H-[EQ]-x(3)-H-x-[LM]-[NQRC]-[GST]-H-[LIVMSTAC](3)-E [The second and third H's bind iron]-

Consensus pattern: [LIVMA]-H-P-[LIVM]-x-[KRQ]-[LIVMF](2)-x-[AP]-H-

[1] Vick B.A., Zimmerman D.C. (In) Biochemistry of plants: A comprehensive treatise, Stumpf P.K., Ed., Vol. 9, pp.53-90, Academic Press, New-York, (1987).

[2] Needleman P., Turk J., Jakschik B.A., Morrison A.R., Lefkowith J.B. Annu. Rev. Biochem. 55:69-102(1986).

[3] Peng Y.L., Shirano Y., Ohta H., Hibino T., Tanaka K., Shibata D. J. Biol. Chem. 269:3755-3761(1994).

[4] Boyington J.C., Gaffney B.J., Amzel L.M. Science 260:1482-1486(1993).

[5] Steczko J., Donoho G.P., Clemens J.C., Dixon J.E., Axelrod B. Biochemistry 31:4053-4057(1992).

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339. Fumarate lyases signature (lyase_1)

A number of enzymes, belonging to the lyase class, for which fumarate is a substrate have been shown [1,2] to share a short conserved sequence around a methionine which is probably involved in the catalytic activity of this type of enzymes. These enzymes are: - Fumarase (EC 4.2.1.2) (fumarate hydratase), which catalyzes the reversible hydration of fumarate to L-malate. There seem to be 2 classes of fumarases: class I are thermolabile dimeric enzymes (as for example: Escherichia coli fumC); class II enzymes are thermostable and tetrameric and are found in prokaryotes (as for example: Escherichia coli fumA and fumB) as well as in eukaryotes. The sequence of the two classes of fumarases are not closely related. - Aspartate ammonia-lyase (EC 4.3.1.1) (aspartase), which catalyzes the reversible conversion of aspartate to fumarate and ammonia. This reaction is analogous to that catalyzed by fumarase, except that ammonia rather than water is involved in the trans-elimination reaction. - Arginosuccinase (EC 4.3.2.1) (argininosuccinate lyase), which catalyzes the formation of arginine and fumarate from argininosuccinate, the last step in the biosynthesis of arginine. - Adenylosuccinase (EC 4.3.2.2) (adenylosuccinate lyase) [3], which catalyzes the eight step in the de novo biosynthesis of purines, the formation of 5'-phosphoribosyl-5-amino-4-imidazolecarboxamide and fumarate from 1-(5- phosphoribosyl)-4-(N-succino-carboxamide). That enzyme can also catalyzes the formation of fumarate and AMP from adenylosuccinate. -

Pseudomonas putida 3-carboxy-cis,cis-muconate cycloisomerase (EC 5.5.1.2) (3-carboxymuconate lactonizing enzyme) (gene pcaB) [4], an enzyme involved in aromatic acids catabolism

Consensus pattern: G-S-x(2)-M-x(2)-K-x-N-

[1] Woods S.A., Shwartzbach S.D., Guest J.R. Biochim. Biophys. Acta 954:14-26(1988).
[2] Woods S.A., Miles J.S., Guest J.R. FEMS Microbiol. Lett. 51:181-186(1988).
[3] Zalkin H., Dixon J.E. Prog. Nucleic Acid Res. Mol. Biol. 42:259-287(1992).
[4] Williams S.E., Woolridge E.M., Ransom S.C., Landro J.A., Babbitt P.C., Kozarich J.W. Biochemistry 31:9768-9776(1992).

340. MCM family signature and profile

Proteins shown to be required for the initiation of eukaryotic DNA replication share a highly conserved domain of about 210 amino-acid residues [1,2,3]. The latter shows some similarities [4] with that of various other families of DNA-dependent ATPases. Eukaryotes seem to possess a family of six proteins that contain this domain. They were first identified in yeast where most of them have a direct role in the initiation of chromosomal DNA replication by interacting directly with autonomously replicating sequences (ARS). They were thus called 'minichromosome maintenance proteins' with gene symbols prefixed by MCM. These six proteins are: - MCM2, also known as cdc19 (in S.pombe) [E1]. - MCM3, also known as DNA polymerase alpha holoenzyme-associated protein P1, RLF beta subunit or ROA. - MCM4, also known as CDC54, cdc21 (in S.pombe) or dpa (in Drosophila). - MCM5, also known as CDC46 or nda4 (in S.pombe). - MCM6, also known as mis5 (in S.pombe). - MCM7, also known as CDC47 or Prolifera (in A.thaliana).This family is also present in archebacteria. In Methanococcus jannaschiithere are four members: MJ0363, MJ0961, MJ1489 and MJECL13.The presence of a putative ATP-binding domain implies that these proteins maybe involved in an ATP-consuming step in the initiation of DNA replication in eukaryotes. As a signature pattern, a perfectly conserved region was selected that represents a special version of the B motif found in ATP-binding proteins.

Consensus pattern: G-[IVT]-[LVAC](2)-[IVT]-D-[DE]-[FL]-[DNST]

[1] Coxon A., Maundrell K., Kearsey S.E. Nucleic Acids Res. 20:5571-5577(1992).
[2] Hu B., Burkhart R., Schulte D., Musahl C., Knippers R. Nucleic Acids Res. 21:5289-5293(1993).
[3] Tye B.-K. Trends Cell Biol. 4:160-166(1994).
[4] Koonin E.V. Nucleic Acids Res. 21:2541-2547(1993).

341. Macrophage migration inhibitory factor family signature (MIF)

A protein called macrophage migration inhibitory factor (MIF) [1] seems to exert an important role in host inflammatory responses. It play a pivotal role in the host response to endotoxic shock and appears to serve as a pituitary "stress" hormone that regulates systemic inflammatory responses. MIF is a secreted protein of 115 residues which is not processed from a larger precursor. D-dopachrome tautomerase [2] is a mammalian cytoplasmic enzyme involved in melanin biosynthesis and that tautomerizes D-dopachrome with concomitant decarboxylation to give 5,6-dihydroxyindole (DHI). It is a protein of 117 residues highly related to MIF. It must be noted that MIF binds glutathione and has been said to be related to glutathione S-transferases. This assertion has been later disproved [3].As a signature pattern for these proteins, a conserved region was selected located in the central section.

Consensus pattern: [DE]-P-C-A-x(3)-[LIVM]-x-S-I-G-x-[LIVM]-G-

[1] Bucala R. Immunol. Lett. 43:23-26(1994).
[2] Odh G., Hindemith A., Rosengren A.-M., Rosengren E., Rorsman H. Biochem. Biophys. Res. Commun. 197:619-624(1993).
[3] Pearson W.R. Protein Sci. 3:525-527(1994).

342. MIP family signature

Recently the sequence of a number of different proteins, that all seem to be transmembrane channel proteins, has been found to be highly related [1 to 4].These proteins are listed below.

- Mammalian major intrinsic protein (MIP). MIP is the major component of lens fiber gap junctions. Gap junctions mediate direct exchange of ions and small molecule from one cell to another. - Mammalian aquaporins [5]. These proteins form water-specific channels that provide the plasma membranes of red cells and kidney proximal and collecting tubules with high permeability to water, thereby permitting water to move in the direction of an osmotic gradient. - Soybean nodulin-26, a major component of the peribacteroid membrane induced during nodulation in legume roots after Rhizobium infection. - Plants tonoplast intrinsic proteins (TIP). There are various isoforms of TIP: alpha (seed), gamma, Rt (root), and Wsi (water-stress induced). These proteins may allow the diffusion of water, amino acids and/or peptides from the tonoplast interior to the cytoplasm. - Bacterial glycerol facilitator protein (gene glpF), which facilitates the movement of glycerol across the cytoplasmic membrane. - Salmonella typhimurium propanediol diffusion facilitator (gene pduF). - Yeast FPS1, a glycerol uptake/efflux facilitator protein. - Drosophila neurogenic protein 'big brain' (bib). This protein may mediate intercellular communication; it may functions by allowing the transport of certain molecules(s) and thereby sending a signal for an exodermal cell to become an epidermoblast instead of a neuroblast. - Yeast hypothetical protein YFL054c. - A hypothetical protein from the pepX region of lactococcus lactis. The MIP family proteins seem to contain six transmembrane segments. Computer analysis shows that these protein probably arose by a tandem, intragenic duplication event from an ancestral protein that contained three transmembrane segments. As a signature pattern a well conserved region was selected which is located in a probable cytoplasmic loop between the second and third transmembrane regions.

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Consensus pattern: [HNQA]-x-N-P-[STA]-[LIVMF]-[ST]-[LIVMF]-[GSTAFY]-

[1] Reizer J., Reizer A., Saier M.H. Jr. CRC Crit. Rev. Biochem. 28:235-257(1993).
[2] Baker M.E., Saier M.H. Jr. Cell 60:185-186(1990).
[3] Pao G.M., Wu L.-F., Johnson K.D., Hoefte H., Chrispeels M.J., Sweet G., Sandal N.N., Saier M.H. Jr. Mol. Microbiol. 5:33-37(1991).
[4] Wistow G.J., Pisano M.M., Chepelinsky A.B. Trends Biochem. Sci. 16:170-171(1991).
[5] Chrispeels M.J., Agre P. Trends Biochem. Sci. 19:421-425(1994).

343. Mandelate racemase / muconate lactonizing enzyme family signatures

Mandelate racemase (EC 5.1.2.2) (MR) and muconate lactonizing enzyme(EC 5.5.1.1) (MLE) are two bacterial enzymes involved in aromatic acid catabolism. They catalyze mechanistically distinct reactions yet they are related at the level of their primary, quaternary (homooctamer) and tertiary structures [1,2].A number of other proteins also seem to be evolutionary related to these two enzymes. These are: - The various plasmid-encoded chloromuconate cycloisomerases (EC 5.5.1.7). - Escherichia coli protein rspA [3], rspA seems to be involved in the degradation of homoserine lactone (HSL) or of one of its metabolite. - Escherichia coli hypothetical protein ycjG. - Escherichia coli hypothetical protein yidU. - A hypothetical protein from Streptomyces ambofaciens [4]. Two signature patterns have been developed for these enzymes; both contain conserved acidic residues. The second pattern contains an aspartate and a glutamate which are ligands for either a magnesium ion (in MR) or a manganese ion (inMLE).

Consensus pattern: A-x-[SAGCN]-[SAG]-[LIVM]-[DEQ]-x-A-[LA]-x-[DE]-[LIA]-x- [GA]-[KRQ]-x(4)-[PSA]-[LIV]-x(2)-L-[LIVMF]-G-
Consensus pattern: [LIVF]-x(2)-D-x-[NH]-x(7)-[ACL]-x(6)-[LIVMF]-x(7)-[LIVM]- E-[DENQ]-P [D and E bind a divalent metal ion]-

[1] Neidhart D.J., Kenyon G.L., Gerlt J.A., Petsko G.A. Nature 347:692-694(1990).
[2] Petsko G.A., Kenyon G.L., Gerlt J.A., Ringe D., Kozarich J.W. Trends Biochem. Sci. 18:372-376(1993).
[3] Huisman G.W., Kolter R. Science 265:537-539(1994).
[4] Schneider D., Aigle B., Leblond P., Simonet J.M., Decaris B. J. Gen. Microbiol. 139:2559-2567(1993).

344. Merozoite Surface Antigen 2 (MSA-2) family

Thomas AW, Carr DA, Carter JM, Lyon JA, Mol Biochem Parasitol 1990;43:211-220.

345. MSP (Major sperm protein) domain.

Major sperm proteins are involved in sperm motility. These proteins oligomerise to form filaments. Partial matches to this domain are also found in other non MSP proteins. These include Swiss:P40075 and Swiss:P34593.

[1] Bullock TL, Roberts TM, Stewart M, J Mol Biol 1996;263:284-296. [2] King KL, Stewart M, Roberts TM, Seavy M, J Cell Sci 1992;101:847-857.

346. (Matrix) Viral matrix protein. Found in Morbillivirus and paramyxovirus, pneumovirus.
Number of members: 105

347. O-methyltransferase (methyltransf)

This family includes a range of O-methyltransferases. These enzymes utilise S-adenosyl methionine.

[1] Keller NP, Dischinger HC, Bhatnagar D, Cleveland TE, Ullah AH, Appl Environ Microbiol 1993;59:479-484.

348. Magnesium chelatase, subunit ChlI

Magnesium-chelatase is a three-component enzyme that catalyses the insertion of Mg2+ into protoporphyrin IX. This is the first unique step in the synthesis of (bacterio)chlorophyll. Due to this, it is thought that Mg-chelatase has an important role in channeling inter- mediates into the (bacterio)chlorophyll branch in response to conditions suitable for photosynthetic growth. ChlI and BchD have molecular weight between 38-42 kDa.

[1] Walker CJ, Willows RD, Biochem J 1997;327:321-333. [2] Petersen BL, Jensen PE, Gibson LC, Stummann BM, Hunter CN, Henningsen KW, J Bacteriol 1998;180:699-704.

349. Plasmid recombination enzyme (Mob_Pre)

With some plasmids, recombination can occur in a site specific manner that is independent of RecA. In such cases, the recombination event requires another protein called

Pre. Pre is a plasmid recombination enzyme. This protein is: also known as Mob (conjugative mobilization).

[1] Priebe SD, Lacks SA, J Bacteriol 1989;171:4778-4784.

350. Monooxygenase

This family includes diverse enzymes that utilise FAD.

[1] Gatti DL, Palfey BA, Lah MS, Entsch B, Massey V, Ballou DP, Ludwig ML, Science 1994;266:110-114.

351. Mov34 family

Members of this family are found in proteasome regulatory subunits, eukaryotic initiation factor 3 (eIF3) subunits and regulators of transcription factors.

[1] Aravind L, Ponting CP, Protein Sci 1998;7:1250-1254. [2] Hershey JW, Asano K, Naranda T, Vornlocher HP, Hanachi P, Merrick WC, Biochimie 1996;78:903-907.

352. Myc amino-terminal region (Myc_N_term)

The myc family belongs to the basic helix-loop-helix leucine zipper class of transcription factors, see HLH. Myc forms a heterodimer with Max, and this complex regulates cell growth through direct activation of genes involved in cell replication [2].

[1] Facchini LM, Penn LZ, FASEB J 1998;12:633-651. [2] Grandori C, Eisenman RN, Trends Biochem Sci 1997;22:177-181.

353. (Metallothio_2) Metallothionein. Members of this family are metallothioneins. These proteins are cysteine rich proteins that bind to heavy metals. Members of this family appear to be closest to Class II metallothioneins, seed metalthio. Number of members: 55

[1] Medline: 98267202. Characterization of gene repertoires at mature stage of citrus fruits through random sequencing and analysis of redundant metallothionein-like genes expressed

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during fruit development. Moriguchi T, Kita M, Hisada S, Endo-Inagaki T, Omura M; Gene 1998;211:221-227.

354. MAGE family

The MAGE (melanoma antigen-encoding gene) family are expressed in a wide variety of tumors but not in normal cells, with the exception of the male germ cells, placenta, and, possibly, cells of the developing embryo. The cellular function of this family is unknown.

[1] McCurdy DK, Tai LQ, Nguyen J, Wang Z, Yang HM, Udar N, Naiem F, Concannon P, Gatti RA; Mol Genet Metab 1998;63:3-13.

355. Malic enzymes signature. Malic enzymes, or malate oxidoreductases, catalyze the oxidative decarboxylation of malate into pyruvate important for a wide range of metabolic pathways. There are three related forms of malic enzyme [1,2,3]: - NAD-dependent malic enzyme (EC 1.1.1.38), which uses preferentially NAD and has the ability to decarboxylate oxaloacetate (OAA). It is found in bacteria and insects. - NAD-dependent malic enzyme (EC 1.1.1.39), which uses preferentially NAD and is unable to decarboxylate OAA. It is found in the mitochondrial matrix of plants and is a heterodimer of highly related subunits. - NADP-dependent malic enzyme (EC 1.1.1.40), which has a preference for NADP and has the ability to decarboxylate OAA. This form has been found in fungi, animals and plants. In mammals, there are two isozymes: one, mitochondrial and the other, cytosolic. Plants also have two isozymes: chloroplastic and cytosolic. There are two other proteins which are closely structurally related to malicenzymes: - Escherichia coli protein sfcA, whose function is not yet known but which could be an NAD or NADP-dependent malic enzyme. - Yeast hypothetical protein YKL029c, a probable malic enzyme. There are three well conserved regions in the enzyme sequences. Two of them seem to be involved in binding NAD or NADP. The significance of the third one, located in the central part of the enzymes, is not yet known. This region has been developed as a signature pattern for these enzymes.

Consensus pattern: F-x-[DV]-D-x(2)-G-T-[GSA]-x-[IV]-x-[LIVMA]-[GAST](2)-[LIVMF](2)-

[1] Artus N.N., Edwards G.E. FEBS Lett. 182:225-233(1985).[2] Loeber G., Infante A.A., Maurer-Fogy I., Krystek E., Dworkin M.B. J. Biol. Chem. 266:3016-3021(1991). [3] Long J.J., Wang J.-L., Berry J.O. J. Biol. Chem. 269:2827-2833(1994).

356. (matrixin)
Matrixins cysteine switch (aka peptidase_M10)

Mammalian extracellular matrix metalloproteinases (EC 3.4.24.-), also known as matrixins [1] (see <PDOC00129>), are zinc-dependent enzymes. They are secreted by cells in an inactive form (zymogen) that differs from the mature enzyme by the presence of an N-terminal propeptide. A highly conserved octapeptide is found two residues downstream of the C-terminal end of the propeptide. This region has been shown to be involved in autoinhibition of matrixins [2,3]; a cysteine within the octapeptide chelates the active site zinc ion, thus inhibiting the enzyme. This region has been called the 'cysteine switch' or 'autoinhibitor region'.

A cysteine switch has been found in the following zinc proteases:

- MMP-1 (EC 3.4.24.7) (interstitial collagenase).
- MMP-2 (EC 3.4.24.24) (72 Kd gelatinase).
- MMP-3 (EC 3.4.24.17) (stromelysin-1).
- MMP-7 (EC 3.4.24.23) (matrilysin).
- MMP-8 (EC 3.4.24.34) (neutrophil collagenase).
- MMP-9 (EC 3.4.24.35) (92 Kd gelatinase).
- MMP-10 (EC 3.4.24.22) (stromelysin-2).
- MMP-11 (EC 3.4.24.-) (stromelysin-3).
- MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).
- MMP-13 (EC 3.4.24.-) (collagenase 3).

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- MMP-14 (EC 3.4.24.-) (membrane-type matrix metalliproteinase 1).
- MMP-15 (EC 3.4.24.-) (membrane-type matrix metalliproteinase 2).
- MMP-16 (EC 3.4.24.-) (membrane-type matrix metalliproteinase 3).
- Sea urchin hatching enzyme (EC 3.4.24.12) (envelysin) [4].
- Chlamydomonas reinhardtii gamete lytic enzyme (GLE) [5].

Consensus patternP-R-C-[GN]-x-P-[DR]-[LIVSAPKQ] [C chelates the zinc ion] Sequences known to belong to this class detected by the pattern ALL, except for cat MMP-7 and mouse MMP-11.

[1] Woessner J. Jr. FASEB J. 5:2145-2154(1991).

□

[2] Sanchez-Lopez R., Nicholson R., Gesnel M.C., Matrisian L.M., Breathnach R. J. Biol. Chem. 263:11892-11899(1988).

[3] Park A.J., Matrisian L.M., Kells A.F., Pearson R., Yuan Z., Navre M. J. Biol. Chem. 266:1584-1590(1991).

[4] Lepage T., Gache C. EMBO J. 9:3003-3012(1990).

[5] Kinoshita T., Fukuzawa H., Shimada T., Saito T., Matsuda Y. Proc. Natl. Acad. Sci. U.S.A. 89:4693-4697(1992).

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357. Vertebrate metallothioneins signature (metalthio)

Metallothioneins (MT) [1,2,3] are small proteins which bind heavy metals such as zinc, copper, cadmium, nickel, etc., through clusters of thiolate bonds. MT's occur throughout the animal kingdom and are also found in higher plants, fungi and some prokaryotes. On the basis of structural relationships MT's have been subdivided into three classes. Class I includes mammalian MT's as well as MT's from crustacean and molluscs, but with clearly related primary structure. Class II groups together MT's from various species such as sea urchins, fungi, insects and cyanobacteria which display none or only very distant correspondence to class I MT's. Class III MT's are atypical polypeptides containing gamma-glutamylcysteinyl units. Vertebrate class I MT's are proteins of 60 to 68 amino acid residues, 20 of these residues are cysteines that bind to 7 bivalent metal ions. As a signature pattern a region that spans 19 residues and which contains seven of the metal-binding cysteines was chosen, this region is located in the N-terminal section of class-I MT's.

Consensus pattern: C-x-C-[GSTAP]-x(2)-C-x-C-x(2)-C-x-C-x(2)-C-x-K-

[1] Hamer D.H. Annu. Rev. Biochem. 55:913-951(1986).
[2] Kagi J.H.R., Schaffer A. Biochemistry 27:8509-8515(1988).
[3] Binz P.-A. Thesis, 1996, University of Zurich.

358. Mitochondrial energy transfer proteins signature (mito_ carr)

Different types of substrate carrier proteins involved in energy transfer are found in the inner mitochondrial membrane [1 to 5]. These are: - The ADP,ATP carrier protein (AAC) (ADP/ATP translocase) which exports ATP into the cytosol and imports ADP into the mitochondrial matrix. The sequence of AAC has been obtained from various mammalian, plant and fungal species. - The 2-oxoglutarate/malate carrier protein (OGCP), which exports 2-oxoglutarate into the cytosol and imports malate or other dicarboxylic acids into the mitochondrial matrix. This protein plays an important role in several metabolic processes such as the malate/aspartate and the oxoglutarate/isocitrate shuttles. - The phosphate carrier protein, which transports phosphate groups from the cytosol into the mitochondrial matrix. - The brown fat uncoupling protein (UCP) which dissipates oxidative energy into heat by transporting protons from the cytosol into the mitochondrial matrix. - The tricarboxylate transport protein (or citrate transport protein) which is involved in citrate-H+/malate exchange. It is important for the bioenergetics of hepatic cells as it provides a carbon source for fatty acid and sterol biosyntheses, and NAD for the glycolytic pathway. - The Grave's disease carrier protein (GDC), a protein of unknown function recognized by IgG in patients with active Grave's disease. - Yeast mitochondrial proteins MRS3 and MRS4. The exact function of these proteins is not known. They suppress a mitochondrial splice defect in the first intron of the COB gene and may act as carriers, exerting their suppressor activity by modulating solute concentrations in the mitochondrion. - Yeast mitochondrial FAD carrier protein (gene FLX1). - Yeast protein ACR1 [6], which seems essential for acetyl-CoA synthetase activity. - Yeast protein PET8. - Yeast protein PMT. - Yeast protein RIM2. - Yeast protein YHM1/SHM1. - Yeast protein YMC1. - Yeast protein YMC2. - Yeast hypothetical proteins YBR291c, YEL006w, YER053c, YFR045w, YHR002w, and YIL006w. - Caenorhabditis elegans hypothetical protein K11H3.3.Two other proteins have been found to belong to this family, yet are not localized in the mitochondrial inner membrane: - Maize amyloplast Brittle-1 protein. This protein, found in the endosperm of kernels, could play a role in amyloplast membrane transport. - Candida boidinii peroxisomal membrane protein PMP47 [7]. PMP47 is an integral membrane protein of the peroxisome and it may play a role as a transporter. These proteins all seem to be evolutionary related. Structurally, they consistof three tandem repeats of a domain of approximately one hundred residues. Each of these domains contains two transmembrane regions. As a signature pattern, one of the most conserved regions in the repeated domain was selected, located just after the first transmembrane region.

Consensus pattern: P-x-[DE]-x-[LIVAT]-[RK]-x-[LRH]-[LIVMFY]-[QGAIVM]-

[1] Klingenberg M. Trends Biochem. Sci. 15:108-112(1990).

[2] Walker J.E. Curr. Opin. Struct. Biol. 2:519-526(1992).

[3] Kuan J., Saier M.H. Jr. CRC Crit. Rev. Biochem. 28:209-233(1993).

[4] Kuan J., Saier M.H. Jr. Res. Microbiol. 144:671-672(1993).

[5] Nelson D.R., Lawson J.E., Klingenberg M., Douglas M.G. J. Mol. Biol. 230:1159-1170(1993).

[6] Palmieri F. FEBS Lett. 346:48-54(1994).

[7] Jank B., Habermann B., Schweyen R.J., Link T.A. Trends Biochem. Sci. 18:427-428(1993).

359. Prokaryotic molybdopterin oxidoreductases signatures (molybdopterin)

A number of different prokaryotic oxidoreductases that require and bind amolybdopterin cofactor have been shown [1,2,3] to share a number of regions of sequence similarity. These enzymes are: - Escherichia coli respiratory nitrate reductase (EC 1.7.99.4). This enzyme complex allows the bacteria to use nitrate as an electron acceptor during anaerobic growth. The enzyme is composed of three different chains: alpha, beta and gamma. The alpha chain (gene narG) is the molybdopterin-binding subunit. Escherichia coli encodes for a second, closely related, nitrate reductase complex which also contains a molybdopterin-binding alpha chain (gene narZ). - Escherichia coli anaerobic dimethyl sulfoxide reductase (DMSO reductase). DMSO reductase is the terminal reductase during anaerobic growth on various sulfoxide and N-oxide compounds. DMSO reductase is composed of three chains: A, B and C. The A chain (gene dmsA) binds molybdopterin. - Escherichia coli biotin sulfoxide reductases (genes bisC and bisZ). This enzyme reduces a spontaneous oxidation product of biotin, BDS, back to biotin. It may serve as a scavenger, allowing the cell to use biotin sulfoxide as a biotin source. - Methanobacterium formicicum formate dehydrogenase (EC 1.2.1.2). The alpha chain (gene fdhA) of this dimeric enzyme binds a molybdopterin cofactor. - Escherichia coli formate dehydrogenases -H (gene fdhF), -N (gene fdnG) and -O (gene fdoG). These enzymes are responsible for the oxidation of formate to carbon dioxide. In addition to molybdopterin, the alpha (catalytic) subunit also contains an active site, selenocysteine. - Wolinella succinogenes polysulfide reductase chain. This enzyme is a component of the phosphorylative electron transport system with polysulfide as the terminal acceptor. It is composed of three chains: A, B and C. The A chain (gene psrA) binds molybdopterin. - Salmonella typhimurium thiosulfate reductase (gene phsA). - Escherichia coli trimethylamine-N-oxide reductase (EC 1.6.6.9) (gene torA) [4]. - Nitrate reductase (EC 1.7.99.4) from Klebsiella pneumoniae (gene nasA), Alcaligenes eutrophus, Escherichia coli, Rhodobacter sphaeroides, Thiosphaera pantotropha (gene napA), and Synechococcus PCC 7942 (gene narB).These proteins range from 715 amino acids (fdhF) to 1246 amino acids (narZ) insize. Three signature patterns for these enzymes were derived. The first is based on a conserved region in the N-terminal section and contains two cysteine residues perhaps involved in binding the molybdopterin cofactor. It should be noted that this region is not present in bisC. The second pattern is derived from a conserved region located in the central part of these enzymes.

Consensus pattern: [STAN]-x-[CH]-x(2,3)-C-[STAG]-[GSTVMF]-x-C-x-[LIVMFYW]-x-[LIVMA]-x(3,4)-[DENQKHT]-

Consensus pattern: [STA]-x-[STAC](2)-x(2)-[STA]-D-[LIVMY](2)-L-P-x-[STAC](2)- x(2)-E-

Consensus pattern: A-x(3)-[GDT]-I-x-[DNQTK]-x-[DEA]-x-[LIVM]-x-[LIVMC]-x- [NS]-x(2)-[GS]-x(5)-A-x-[LIVM]-[ST]-

[1] Wootton J.C., Nicolson R.E., Cock J.M., Walters D.E., Burke J.F., Doyle W.A., Bray R.C. Biochim. Biophys. Acta 1057:157-185(1991).

[2] Bilous P.T., Cole S.T., Anderson W.F., Weiner J.H. Mol. Microbiol. 2:785-795(1988).

[3] Trieber C.A., Rothery R.A., Weiner J.H. J. Biol. Chem. 269:7103-7109(1994).

[4] Mejean V., Lobbi-Nivol C., Lepelletier M., Giordano G., Chippaux M., Pascal M.-C. Mol. Microbiol. 11:1169-1179(1994).

360. Bacterial mutT domain signature

The bacterial mutT protein is involved in the GO system [1] responsible for removing an oxidatively damaged form of guanine (8-hydroxyguanine or7,8-dihydro-8-oxoguanine) from DNA and the nucleotide pool. 8-oxo-dGTP is inserted opposite to dA and dC residues of template DNA with almost equal efficiency thus leading to A.T to G.C transversions. MutT specifically degrades 8-oxo-dGTP to the monophosphate with the concomitant release of pyrophosphate. MutT is a small protein of about 12 to 15 Kd. It has been shown [2,3] that a region of about 40 amino acid residues, which is found in the N-terminal part of mutT, can also be found in a variety of other prokaryotic, viral, and eukaryotic proteins. These proteins are:

- Streptomyces pneumoniae mutX.
- A mutT homolog from plasmid pSAM2 of Streptomyces ambofaciens.
- Bartonella bacilliformis invasion protein A (gene invA).
- Escherichia coli dATP pyrophosphohydrolase.
- Protein D250 from African swine fever viruses.
- Proteins D9 and D10 from a variety of poxviruses.
- Mammalian 7,8-dihydro-8-oxoguanine triphosphatase (EC 3.1.6.-) [4].
- Mammalian diadenosine 5',5'''-P1,P4-tetraphosphate asymmetrical hydrolase (Ap4Aase) (EC 3.6.1.17) [5], which cleaves A-5'-PPPP-5'A to yield AMP and ATP.
- A protein encoded on the antisense RNA of the basic fibroblast growth factor gene in higher vertebrates.
- Yeast protein YSA1.
- Escherichia coli hypothetical protein yfaO.

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- Escherichia coli hypothetical protein ygdU and HI0901, the corresponding Haemophilus influenzae protein.
- Escherichia coli hypothetical protein yjaD and HI0432, the corresponding Haemophilus influenzae protein.
- Escherichia coli hypothetical protein yrfE.
- Bacillus subtilis hypothetical protein yqkG.
- Bacillus subtilis hypothetical protein yzgD.
- Yeast hypothetical protein YGL067w.

It is proposed [2] that the conserved domain could be involved in the active center of a family of pyrophosphate-releasing NTPases. As a signature pattern the core region of the domain was selected; it contains four conserved glutamate residues.

Consensus pattern: G-x(5)-E-x(4)-[STAGC]-[LIVMAC]-x-R-E-[LIVMFT]-x-E-E-

[1] Michaels M.L., Miller J.H. J. Bacteriol. 174:6321-6325(1992).
[2] Koonin E.V. Nucleic Acids Res. 21:4847-4847(1993).
[3] Mejean V., Salles C., Bullions M.J., Bessman M.J., Claverys J.-P. Mol. Microbiol. 11:323-330(1994).
[4] Sakumi K., Furuichi M., Tsuzuki T., Kakuma T., Kawabata S., Maki H., Sekiguchi M. J. Biol. Chem. 268:23524-23530(1993).
[5] Thorne N.M.H., Hankin S., Wilkinson M.C., Nunez C., Barraclough R., McLennan A.G. Biochem. J. 311:717-721(1995).

361. Myb DNA-binding domain repeat signatures

The retroviral oncogene v-myb , and its cellular counterpart c-myb, encodenuclear DNA-binding proteins that specifically recognize the sequence YAAC(G/T)G [1]. The myb family also includes the following proteins: - Drosophila D-myb [2]. - Vertebrate myb-like proteins A-myb and B-myb [3]. - Maize C1 protein, a trans-acting factor which controls the expression of genes involved in anthocyanin biosynthesis. - Maize P protein [4], a trans-acting factor which regulates the biosynthetic pathway of a flavonoid-derived pigment in certain floral tissues. - Arabidopsis thaliana protein GL1 [5], required for the initiation of differentiation of leaf hair cells (trichomes). - A number of myb/c1-related proteins in maize and barley, whose roles are not yet known [4]. - Yeast BAS1 [7], a transcriptional activator

for the HIS4 gene. - Yeast REB1 [8], which recognizes sites within both the enhancer and the promoter of rRNA transcription, as well as upstream of many genes transcribed by RNA polymerase II. - Fission yeast cdc5, a possible transcription factor whose activity is required for cell cycle progression and growth during G2. - Fission yeast myb1, which regulates telomere length and function. - Yeast hypothetical protein YMR213w.One of the most conserved regions in all of these proteins is a domain of 160amino acids. It consists of three tandem repeats of 51 to 53 amino acids. In myb, this repeat region has been shown [9] to be involved in DNA-binding. The major part of the first repeat is missing in retroviral v-myb sequences and in plant myb-related proteins. Yeast REB1 differs from the other proteins in this family in having a single myb-like domain. As shown in the following schematic representation, two signature patterns for myb-like domains were developed; the first is located in the N-terminal section, the second spans the C-terminal extremity of the domain.
xxxxxxxxxWxxxEDxxxxxxxxxxxxxxWxxIxxxxxxRxxxxxxxxWxxxx **********
************************'*' : Position of the patterns.

Consensus pattern: W-[ST]-x(2)-E-[DE]-x(2)-[LIV]-
Consensus pattern: W-x(2)-[LI]-[SAG]-x(4,5)-R-x(8)-[YW]-x(3)-[LIVM]-
Note: this pattern detects the three copies of the domain in myb, d-myb, A-myb and B-myb; the second of the two complete copies of plant myb-related proteins, and the last two copies of yeast BAS1

[1] Biednkapp H., Borgmeyer U., Sippel A.E., Klempnauer K.-H. Nature 335:835-837(1988).
[2] Peters C.W.B., Sippel A.E., Vingron M., Klempnauer K.-H. EMBO J. 6:3085-3090(1987).
[3] Nomura N., Takahashi M., Matsui M., Ishii S., Date T., Sasamoto S., Ishizaki R. Nucleic Acids Res. 16:11075-11090(1988).
[4] Grotewold E., Athma P., Peterson T. Proc. Natl. Acad. Sci. U.S.A. 88:4587-4591(1991).
[5] Oppenheimer D.G., Herman P.L., Sivakumaran S., Esch J., Marks M.D. Cell 67:483-493(1991).
[6] Marocco A., Wissenbach M., Becker D., Paz-Ares J., Saedler H., Salamini F., Rohde W. Mol. Gen. Genet. 216:183-187(1989).
[7] Tice-Baldwin K., Fink G.R., Arndt K.T. Science 246:931-935(1989).

[8] Ju Q., Morrow B.E., Warner J.R. Mol. Cell. Biol. 10:5226-5234(1990).

[9] Klempnauer K.-H., Sippel A.E. EMBO J. 6:2719-2725(1987).

362. NAD-dependent glycerol-3-phosphate dehydrogenase signature

NAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) (GPD) catalyzes the reversible reduction of dihydroxyacetone phosphate to glycerol-3- phosphate. It is a eukaryotic cytosolic homodimeric protein of about 40 Kd. As a signature pattern a glycine-rich region that is probably [1] involved in NAD-binding was selected.

Consensus pattern: G-[AT]-[LIVM]-K-[DN]-[LIVM](2)-A-x-[GA]-x-G-[LIVMF]-x- [DE]-G-[LIVM]-x-[LIVMFYW]-G-x-N-

[1] Otto J., Argos P., Rossmann M.G. Eur. J. Biochem. 109:325-330(1980).

363. Nucleosome assembly protein (NAP)

It is thought that NAPs may be involved in regulating gene expression as a result of histone accessibility [1].

[1] Rodriguez P, Munroe D, Prawitt D, Chu LL, Bric E, Kim J, Reid LH, Davies C, Nakagama H, Loebbert R, Winterpacht A, Petruzzi MJ, Higgins MJ, Nowak N, Evans G, Shows T, Weissman BE, Zabel B, Housman DE, Pelletier J, Genomics 1997;44:253-265. [2] Schnieders F, Dork T, Arnemann J, Vogel T, Werner M, Schmidtke J; Hum Mol Genet 1996;5:1801-1807.

364. NB-ARC domain

van der Biezen EA, Jones JD, Curr Biol 1998;8:226-227.

365. Nucleoside diphosphate kinases active site

Nucleoside diphosphate kinases (EC 2.7.4.6) (NDK) [1] are enzymes required for the synthesis of nucleoside triphosphates (NTP) other than ATP. They provide NTPs for nucleic acid synthesis, CTP for lipid synthesis, UTP for polysaccharide synthesis and GTP for protein elongation, signal transduction and microtubule polymerization. In eukaryotes, there seems to be a small family of NDK isozymes each of which acts in a different subcellular compartment and/or has a distinct biological function. Eukaryotic NDK isozymes are hexamers of two highly related chains (Aand B) [2]. By random association (A6, A5B...AB5, B6), these two kinds of chain form isoenzymes differing in their isoelectric point. NDK are proteins of 17 Kd that act via a ping-pong mechanism in which a histidine residue is phosphorylated, by transfer of the terminal phosphate group from ATP. In the presence of magnesium, the phosphoenzyme can transfer its phosphate group to any NDP, to produce an NTP.NDK isozymes have been sequenced from prokaryotic and eukaryotic sources. It has also been shown [3] that the Drosophila awd (abnormal wing discs) protein, is a microtubule-associated NDK. Mammalian NDK is also known as metastasis inhibition factor nm23.The sequence of NDK has been highly conserved through evolution. There is a single histidine residue conserved in all known NDK isozymes, which is involved in the catalytic mechanism [2]. Our signature pattern contains this residue.

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Consensus pattern: N-x(2)-H-[GA]-S-D-[SA]-[LIVMPKNE] [H is the putative active site residue]-

[1] Parks R., Agarwal R. (In) The Enzymes (3rd edition) 8:307-334(1973).
[2] Gilles A.-M., Presecan E., Vonica A., Lascu I. J. Biol. Chem. 266:8784-8789(1991).
[3] Biggs J., Hersperger E., Steeg P.S., Liotta L.A., Shearn A. Cell 63:933-940(1990).

366. Nitrite and sulfite reductases iron-sulfur/siroheme-binding site (NIR_SIR)

Nitrite reductases (NiR) [1] catalyze the reduction of nitrite into ammonium, the second step in the assimilation of nitrate. There are two types of NiR: the higher plant chloroplastic form of NiR (EC 1.7.7.1) is a monomeric protein that uses reduced ferredoxin as the electron donor; while fungal and bacterial NiR (EC 1.6.6.4) are homodimeric proteins that uses NAD(P)H as the electron donor. Both forms of NiR contain a siroheme-Fe and iron-sulfur centers. Sulfite reductase (NADPH) (EC 1.8.1.2) (SIR) [2] is the bacterial enzyme that catalyzes the reduction of sulfite to sulfide. SIR is an oligomeric enzyme with a subunit composition of alpha(8)-beta(4), the alpha component is a flavoprotein (SIR-FP), while the beta component is a siroheme, iron-sulfurprotein (SIR-HP).Sulfite reductase (ferredoxin) (EC 1.8.7.1) [3] is a cyanobacterial and plant monomeric enzyme that also catalyzes the reduction of sulfite to sulfide. Anaerobic sulfite reductase (EC 1.8.1.-) (ASR) [4], a bacterial enzyme that catalyzes the NADH-dependent reduction of sulfite to sulfide. ASR is an oligomeric enzyme composed of three different subunits. The C component (geneasrC) seems to be a siroheme, iron-sulfur protein. These enzymes share a region of sequence similarity in their C-terminal half; this region which spans about 80 amino acids includes four conserved cysteine residues. Two of the Cys are grouped together at the beginning of the domain, and the two others are grouped in the middle of the domain. The cysteines are involved in the binding of the iron-sulfur center; the last one also binds the siroheme group [2]. A signature pattern from the region around the second cluster of cysteines was derived.

Consensus pattern: [STV]-G-C-x(3)-C-x(6)-[DE]-[LIVMF]-[GAT]-[LIVMF] [The two C's are ison-sulfur ligands]-

[1] Campbell W.H., Kinghorn J.R. Trends Biochem. Sci. 15:315-319(1990).

[2] Crane B.R., Siegel L.M., Getzoff E.D. Science 270:59-67(1995).

[3] Gisselmann G., Klausmeier P., Schwenn J.D. Biochim. Biophys. Acta 1144:102-106(1993).

[4] Huang C.J., Barrett E.L. J. Bacteriol. 173:1544-1553(1991).

367. (NMT) Myristoyl-CoA:protein N-myristoyltransferase signatures. Myristoyl-CoA: protein N-myristoyltransferase (EC 2.3.1.97) (Nmt) [1] is the enzyme responsible for transferring a myristate group on the N-terminal glycine of a number of cellular eukaryotic and viral proteins. Nmt is a monomeric protein of about 50 to 60 Kd whose sequence appears to be well conserved. Two highly conserved regions have been developed as signature patterns. The first one is located in the central section, the second in the C-terminal part.

Consensus pattern: E-I-N-F-L-C-x-H-K-

Consensus pattern: K-F-G-x-G-D-G-

09555350.071000

[1] Rudnick D.A., McWherter C.A., Gokel G.W., Gordon J.I. Adv. Enzymol. 67:375-430(1993).

368. ADP-glucose pyrophosphorylase signatures (NTP_transferase)

ADP-glucose pyrophosphorylase (glucose-1-phosphate adenylyltransferase) [1,2](EC 2.7.7.27) catalyzes a very important step in the biosynthesis of alpha 1,4-glucans (glycogen or starch) in bacteria and plants: synthesis of the activated glucosyl donor, ADP-glucose, from glucose-1-phosphate and ATP.ADP-glucose pyrophosphorylase is a tetrameric allosterically regulated enzyme. It is a homotetramer in bacteria while in plant chloroplasts and amyloplasts, it is a heterotetramer of two different, yet evolutionary related, subunits. There are a number of conserved regions in the sequence of bacterial and plant ADP-glucose pyrophosphorylase subunits. Three of these regions were selected as signature patterns. The first two are N-terminal and have been proposed to be part of the allosteric and/or substrate-binding sites in the Escherichia coli enzyme (gene glgC). The third pattern corresponds to a conserved region in the central part of the enzymes.

Consensus pattern: [AG]-G-G-x-G-[STK]-x-L-x(2)-L-[TA]-x(3)-A-x-P-A-[LV] -
Consensus pattern: W-[FY]-x-G-[ST]-A-[DNSH]-[AS]-[LIVMFYW]-
Consensus pattern: [APV]-[GS]-M-G-[LIVMN]-Y-[IVC]-[LIVMFY]-x(2)-[DENPHK] -

[1] Nakata P.A., Greene T.W., Anderson J.M., Smith-White B.J., Okita T.W., Preiss J. Plant Mol. Biol. 17:1089-1093(1991).
[2] Preiss J., Ball K., Hutney J., Smith-White B.J., Li. L., Okitsa T.W. Pure Appl. Chem. 63:535-544(1991).

369. Sodium/hydrogen exchanger family

Na/H antiporters are key transporters in maintaining the pH of actively metabolizing cells. The molecular mechanisms of antiport are unclear.

These antiporters contain 10-12 transmembrane regions (M) at the amino-terminus and a large cytoplasmic region at the carboxyl

09555306.061502

terminus. The transmembrane regions M3-M12 share identity with other members of the family. The M6 and M7 regions are highly conserved. Thus, this is thought to be the region that is involved in the transport of sodium and hydrogen ions. The cytoplasmic region has little similarity throughout the family.

[1] Dibrov P, Fliegel L; FEBS Lett 1998;424:1-5. [2] Orlowski J, Grinstein S; J Biol Chem 1997;272:22373-22376.[3] Numata M, Petrecca K, Lake N, Orlowski J; J Biol Chem 1998;273:6951-6959.

09585372.091400

370. Sodium:sulfate symporter family signature (Na_sulph_symp)

Integral membrane proteins that mediate the intake of a wide variety of molecules with the concomitant uptake of sodium ions (sodium symporters) canbe grouped, on the basis of sequence and functional similarities into a number of distinct families. One of these families currently consists of the following proteins: - Mammalian sodium/sulfate cotransporter [1]. - Mammalian renal sodium/dicarboxylate cotransporter [2], which transports succinate and citrate. - Mammalian intestinal sodium/dicarboxylate cotransporter. - Chlamydomonas reinhardtii putative sulfur deprivation response regulator SAC1 [3]. - Caenorhabditis elegans hypothetical proteins B0285.6, F31F6.6, K08E5.2 and R107.1. - Escherichia coli hypothetical protein yfbS. - Haemophilus influenzae hypothetical protein HI0608. - Synechocystis strain PCC 6803 hypothetical protein sll0640. - Methanococcus jannaschii hypothetical protein MJ0672.These transporters are proteins of from 430 to 620 amino acids which are highly hydrophobic and which probably contain about 12 transmembrane regions. As a signature pattern, a conserved region was selected which is located in or near the penultimate transmembrane region.

Consensus pattern: [STACP]-S-x(2)-F-x(2)-P-[LIVM]-[GSA]-x(3)-N-x-[LIVM]-V-

[1] Markovich D., Forgo J., Stange G., Biber J., Murer H. Proc. Natl. Acad. Sci. U.S.A. 90:8073-8077(1993).

[2] Pajor A.M. Am. J. Physiol. 270:642-648(1996).

[3] Davies J.P., Yildiz F.H., Grossman A. EMBO J. 15:2150-2159(1996).

371. NifU-like domain

This is an alignment of the carboxy-terminal domain. This is the only common region between the NifU protein from nitrogen-fixing bacteria and rhodobacterial species. The biochemical function of NifU is unknown [1].

Ouzounis C, Bork P, Sander C, Trends Biochem Sci 1994;19:199-200.

372. Nitrilases / cyanide hydratase signatures

Nitrilases (EC 3.5.5.1) are enzymes that convert nitriles into their corresponding acids and ammonia. They are widespread in microbes as well as in plants where they convert indole-3-acetonitrile to the hormone indole-3-acetic acid. A conserved cysteine has been shown [1,2] to be essential for enzyme activity; it seems to be involved in a nucleophilic attack on the nitrile carbon atom. Cyanide hydratase (EC 4.2.1.66) converts HCN to formamide. In phytopathogenic fungi, it is used to avoid the toxic effect of cyanide released by wounded plants [3]. The sequence of cyanide hydrolase is evolutionary related to that of nitrilases. Yeast hypothetical proteins YIL164c and YIL165c also belong to this family. As signature patterns for these enzymes, two conserved regions were selected. The first is located in the N-terminal section while the second, which contains the active site cysteine, is located in the central section.

Consensus pattern: G-x(2)-[LIVMFY](2)-x-[IF]-x-E-x(2)-[LIVM]-x-G-Y-P-

Consensus pattern: G-[GAQ]-x(2)-C-[WA]-E-[NH]-x(2)-[PST]-[LIVMFYS]-x-[KR] [C is the active site residue]-

[1] Kobayashi M., Izui H., Nagasawa T., Yamada H. Proc. Natl. Acad. Sci. U.S.A. 90:247-251(1993).

[2] Kobayashi M., Komeda H., Yanaka N., Nagasawa T., Yamada H. J. Biol. Chem. 267:20746-20751(1992).

[3] Wang P., Vanetten H.D. Biochem. Biophys. Res. Commun. 187:1048-1054(1992).

373. NusB family

The NusB protein is involved in the regulation of rRNA biosynthesis by transcriptional antitermination.

Huenges M, Rolz C, Gschwind R, Peteranderl R, Berglechner F, Richter G, Bacher A, Kessler H,Gemmecker G, EMBO J 1998;17:4092-4100.

374. (Neur Chan) Neurotransmitter-gated ion-channels signature

Neurotransmitter-gated ion-channels [1,2,3,4] provide the molecular basis for rapid signal transmission at chemical synapses. They are post-synapticoligomeric transmembrane complexes that transiently form a ionic channel upon the binding of a specific neurotransmitter. Presently, the sequence of subunits from five types of neurotransmitter-gated receptors are known: - The nicotinic acetylcholine receptor (AchR), an excitatory cation channel. In the motor endplates of vertebrates, it is composed of four different subunits (alpha, beta, gamma and delta or epsilon) with a molar stoichiometry of 2:1:1:1. In neurones, the AchR receptor is composed of two different types of subunits: alpha and non-alpha (also called beta). Nicotinic AchRs are also found in invertebrates. - The glycine receptor, an inhibitory chloride ion channel. The glycine receptor is a pentamer composed of two different subunits (alpha and beta). - The gamma-aminobutyric-acid (GABA) receptor, which is also an inhibitory chloride ion channel. The quaternary structure of the GABA receptor is complex; at least four classes of subunits are known to exist (alpha, beta, gamma, and delta) and there are many variants in each class (for example: six variants of the alpha class have already been sequenced). - The serotonin 5HT3 receptor. Serotonin is a biogenic hormone that functions as a neurotransmitter, a hormone and a mitogen. There are seven major groups of serotonin receptors; six of these groups (5HT1, 5HT2, and 5HT4 to 5HT7) transduce extracellular signal by activating G proteins, while 5HT3 is a ligand-gated cation-specific ion channel which, when activated causes fast, depolarizing responses in neurons. - The glutamate receptor, an excitatory cation channel. Glutamate is the main excitatory neurotransmitter in the brain. At least three different types of glutamate receptors have been described and are named according to their selective agonists (kainate, N-methyl-D-aspartate (NMDA) and quisqualate).All known sequences of subunits from neurotransmitter-gated ion-channels are structurally related. They are composed of a large extracellular glycosylated N-terminal ligand-binding domain, followed by three hydrophobic transmembrane regions which form the ionic channel, followed by an intracellular region of variable length. A fourth hydrophobic region is found at the C-terminal of the sequence. The sequence of subunits from the AchR, GABA, 5HT3, and Gly receptors are clearly evolutionary related and share many regions of sequence similarities. These sequence similarities are either absent or very weak in the Glu receptors. In the N-terminal extracellular domain of AchR/GABA/5HT3/Gly receptors, there are two conserved cysteine residues, which, in AchR, have been shown to form a disulfide bond essential to the tertiary structure of the receptor. A number of amino acids between the two disulfide-bonded cysteines are also conserved. Therefore this region was used as a signature pattern for this subclass of proteins.

Consensus pattern: C-x-[LIVMFQ]-x-[LIVMF]-x(2)-[FY]-P-x-D-x(3)-C [The two C's are linked by a disulfide bond]-

[1] Stroud R.M., McCarthy M.P., Shuster M. Biochemistry 29:11009-11023(1990).
[2] Betz H. Neuron 5:383-392(1990).
[3] Dingledine R., Myers S.J., Nicholas R.A. FASEB J. 4:2632-2645(1990).
[4] Barnard E.A. Trends Biochem. Sci. 17:368-374(1992).

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375. Orotidine 5'-phosphate decarboxylase active site

Orotidine 5'-phosphate decarboxylase (EC 4.1.1.23) (OMPdecase) [1,2] catalyzes the last step in the de novo biosynthesis of pyrimidines, the decarboxylation of OMP into UMP. In higher eukaryotes OMPdecase is part, with orotatephosphoribosyltransferase, of a bifunctional enzyme, while the prokaryotic and fungal OMPdecases are monofunctional protein. Some parts of the sequence of OMPdecase are well conserved across species. The best conserved region is located in the N-terminal half of OMPdecases and is centered around a lysine residue which is essential for the catalytic function of the enzyme. This region has been developed as a signature pattern.

Consensus pattern: [LIVMFTA]-[LIVMF]-x-D-x-K-x(2)-D-I-[GP]-x-T-[LIVMTA] [K is the active site residue]-

[1] Jacquet M., Guilbaud R., Garreau H. Mol. Gen. Genet. 211:441-445(1988).
[2] Kimsey H.H., Kaiser D. J. Biol. Chem. 267:819-824(1992).

376. ATP synthase delta (OSCP) subunit signature

ATP synthase (proton-translocating ATPase) (EC 3.6.1.34) [1,2] is a component of the cytoplasmic membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The ATPase complex is composed of an oligomeric transmembrane sector, called CF(0), which acts as a proton channel, and a catalytic core, termed coupling factor CF(1).

One of the subunits of the ATPase complex, known as subunit delta in bacteria and chloroplasts or the Oligomycin Sensitivity Conferral Protein (OSCP) in mitochondria, seems to be part of the stalk that links CF(0) to CF(1). It either transmits conformational changes from CF(0) into CF(1) or is involved in proton conduction [3].

The different delta/OSCP subunits are proteins of approximately 200 amino-acid residues - once the transit peptide has been removed in the chloroplast and mitochondrial forms - which show only moderate sequence homology.

The signature pattern used to detect ATPase delta/OSCP subunits is based on a conserved region in the C-terminal section of these proteins.

Consensus pattern: [LIVM]-x-[LIVMFYT]-x(3)-[LIVMT]-[DENQK]-x(2)-[LIVM]-x-[GSA]-G-[LIVMFYGA]-x-[LIVM]-[KRHENQ]-x-[GSEN]

[1] Futai M., Noumi T., Maeda M. Annu. Rev. Biochem. 58:111-136(1989).

[2] Senior A.E. Physiol. Rev. 68:177-231(1988).

[3] Engelbrecht S., Junge W. Biochim. Biophys. Acta 1015:379-390(1990).

377. Aspartate and ornithine carbamoyltransferases signature

Aspartate carbamoyltransferase (EC 2.1.3.2) (ATCase) catalyzes the conversion of aspartate and carbamoyl phosphate to carbamoylaspartate, the second step in the de novo biosynthesis of pyrimidine nucleotides [1]. In prokaryotes ATCase consists of two subunits: a catalytic chain (gene pyrB) and a regulatory chain (gene pyrI), while in eukaryotes it is a domain in a multifunctional enzyme (called URA2 in yeast, rudimentary in Drosophila, and CAD in mammals [2]) that also catalyzes other steps of the biosynthesis of pyrimidines.

Ornithine carbamoyltransferase (EC 2.1.3.3) (OTCase) catalyzes the conversion of ornithine and carbamoyl phosphate to citrulline. In mammals this enzyme participates in the urea cycle [3] and is located in the mitochondrial matrix. In prokaryotes and eukaryotic microorganisms it is involved in the biosynthesis of arginine. In some bacterial species it is also involved in the degradation of arginine [4] (the arginine deaminase pathway).

It has been shown [5] that these two enzymes are evolutionary related. The predicted secondary structure of both enzymes are similar and there are some regions of sequence similarities. One of these regions includes three residues which have been shown, by crystallographic studies [6], to be implicated in binding the phosphoryl group of carbamoyl phosphate.

This region was selected as a signature for these enzymes.

Consensus pattern: F-x-[EK]-x-S-[GT]-R-T[S, R, and the 2nd T bind carbamoyl phosphate]

-Note: the residue in position 3 of the pattern allows to distinguish between an ATCase (Glu) and an OTCase (Lys).

[1] Lerner C.G., Switzer R.L. J. Biol. Chem. 261:11156-11165(1986).

[2] Davidson J.N., Chen K.C., Jamison R.S., Musmanno L.A., Kern C.B. BioEssays 15:157-164(1993).

[3] Takiguchi M., Matsubasa T., Amaya Y., Mori M. BioEssays 10:163-166(1989).

[4] Baur H., Stalon V., Falmagne P., Luethi E., Haas D. Eur. J. Biochem. 166:111-117(1987).

[5] Houghton J.E., Bencini D.A., O'Donovan G.A., Wild J.R. Proc. Natl. Acad. Sci. U.S.A. 81:4864-4868(1981).

[6] Ke H.-M., Honzatko R.B., Lipscomb W.N. Proc. Natl. Acad. Sci. U.S.A. 81:4037-4040(1984).

378. Oleosins signature

Oleosins [1] are the proteinaceous components of plants' lipid storage bodies called oil bodies. Oil bodies are small droplets (0.2 to 1.5 mu-m in diameter) containing mostly triacylglycerol that are surrounded by a phospholipid/ oleosin annulus. Oleosins may have a structural role in stabilizing the lipid body during dessication of the seed, by preventing coalescence of the oil. They may also provide recognition signals for specific lipase anchorage in lipolysis during seedling growth. Oleosins are found in the monolayer lipid/ water interface of oil bodies and probably interact with both the lipid and phospholipid moieties.

Oleosins are proteins of 16 Kd to 24 Kd and are composed of three domains: an N-terminal hydrophilic region of variable length (from 30 to 60 residues); a central hydrophobic domain of about 70 residues and a C-terminal amphipathic region of variable length (from 60 to 100 residues). The central hydrophobic domain is proposed to be made up of beta-strand structure and to interact with the lipids [2]. It is the only domain whose sequence is conserved and therefore a section from that domain was selected as a signature pattern.

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Consensus pattern: [AG]-[ST]-x(2)-[AG]-x(2)-[LIVM]-[SAD]-T-P-[LIVMF](4)-F-S-P-[LIVM](3)-P-A

[1] Murphy D.J., Keen J.N., O'Sullivan J.N., Au D.M.Y., Edwards E.-W., Jackson P.J., Cummins I., Gibbons T., Shaw C.H., Ryan A.J. Biochim. Biophys. Acta 1088:86-94(1991).
[2] Tzen J.T.C., Lie G.C., Huang A.H.C. J. Biol. Chem. 267:15626-15634(1992).

379. (Orbi VP5) Orbivirus outer capsid protein VP5

This paper shows the location of the different capsid proteins and their relation to each other.

[1] Schoehn G, Moss SR, Nuttall PA, Hewat EA; Virology 1997;235:191-200.

380. Orn/DAP/Arg decarboxylases family 2 signatures

Pyridoxal-dependent decarboxylases acting on ornithine, lysine, arginine and related substrates can be classified into two different families on the basis of sequence similarities [1,2,3]. The second family consists of:

- Eukaryotic ornithine decarboxylase (EC 4.1.1.17) (ODC). ODC catalyzes the transformation of ornithine into putrescine.
- Prokaryotic diaminopimelic acid decarboxylase (EC 4.1.1.20) (DAPDC). DAPDC catalyzes the conversion of diaminopimelic acid into lysine; the last step in the biosynthesis of lysine.
- Pseudomonas syringae pv. tabaci protein tabA. tabA is probably involved in the biosynthesis of tabtoxin and is highly similar to DAPDC.
- Bacterial and plant biosynthetic arginine decarboxylase (EC 4.1.1.19) (ADC). ADC catalyzes the transformation of arginine into agmatine, the first step in the biosynthesis of putrescine from arginine.

The above proteins, while most probably evolutionary related, do not share extensive regions of sequence similarities. Two of the conserved regions were selected as signature patterns. The first pattern contains a conserved lysine residue which is known, in mouse ODC [4], to be the site of attachment of the pyridoxal-phosphate group. The second pattern contains a stretch of three consecutive glycine residues and has been proposed to be part of a substrate-binding region [5].

These enzymes are collectively known as group IV decarboxylases [3].

Consensus pattern: [FY]-[PA]-x-K-[SACV]-[NHCLFW]-x(4)-[LIVMF]-[LIVMTA]-x(2)-[LIVMA]-x(3)-[GTE] [K is the pyridoxal-P attachment site]

Consensus pattern: [GS]-x(2,6)-[LIVMSCP]-x(2)-[LIVMF]-[DNS]-[LIVMCA]-G-G-G-[LIVMFY]-[GSTPCEQ]

[1] Bairoch A. Unpublished observations (1993).

[2] Martin C., Cami B., Yeh P., Stragier P., Parsot C., Patte J.-C. Mol. Biol. Evol. 5:549-559(1988).

[3] Sandmeier E., Hale T.I., Christen P. Eur. J. Biochem. 221:997-1002(1994).

[4] Poulin R., Lu L., Ackermann B., Bey P., Pegg A.E. J. Biol. Chem. 267:150-158(1992).

[5] Moore R.C., Boyle S.M. J. Bacteriol. 172:4631-4640(1990).

381. Osteopontin signature

Osteopontin is an acidic phosphorylated glycoprotein of about 40 Kd which is abundant in the mineral matrix of bones and which binds tightly to hydroxyapatite [1,2,3]. It is suggested that osteopontin might function as a cell attachment factor and could play a key role in the adhesion of osteoclasts to the mineral matrix of bone.

Osteopontin-K is a kidney protein which is highly similar to osteopontin and probably also involved in cell-adhesion.

As a signature pattern a highly conserved region located at the N-terminal extremity of the mature protein was selected.

Consensus pattern: [KQ]-x-[TA]-x(2)-[GA]-S-S-E-E-K

[1] Butler W.T. Connect. Tissue Res. 23:123-36(1989).
[2] Gorski J.P. Calcif. Tissue Int. 50:391-396(1992).
[3] Denhardt D.T., Guo X. FASEB J. 7:1475-1482(1993).

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382. Oxysterol-binding protein family signature

A number of eukaryotic proteins that seem to be involved with sterol synthesis and/or its regulation have been found [1] to be evolutionary related:

- Mammalian oxysterol-binding protein (OSBP). A protein of about 800 amino-acid residues that binds a variety of oxysterols: oxygenated derivatives of cholesterol. OSBP seems to play a complex role in the regulation of sterol metabolism.
- Yeast proteins HES1 and KES1; highly related proteins of 434 residues that seem to play a role in ergosterol synthesis.
- Yeast OSH1, a protein of 859 residues that also plays a role in ergosterol synthesis. - Yeast hypothetical protein YHR001w (437 residues).
- Yeast hypothetical protein YHR073w (996 residues).

- Yeast hypothetical protein YKR003w (448 residues).

All these proteins contain a moderately conserved domain of about 250 residues located in the C-terminal half of OBSP, OSH1 and YHR073w and in the central section of the other proteins. As a signature pattern, the best conserved part was selected of this domain, a region that contains a conserved pentapeptide.

Consensus pattern: E-[KQ]-x-S-H-[HR]-P-P-x-[STACF]-A

[1] Jiang B., Brown J.L., Sheraton J., Fortin N., Bussey H. Yeast 10:341-353(1994).

09555326.051500

383. FMN oxidoreductase

384. Oxidoreductase FAD/NAD-binding domain

Number of members: 250

[1]

Medline: 92084635

The sequence of squash NADH:nitrate reductase and its relationship to the sequences of other flavoprotein oxidoreductases. A family of flavoprotein pyridine nucleotide cytochrome reductases.

Hyde GE, Crawford NM, Campbell W;

J Biol Chem 1991;266:23542-23547.

[2]Medline: 95111952

Crystal structure of the FAD-containing fragment of corn nitrate reductase at 2.5 A resolution: relationship to other flavoprotein reductases.

Lu G, Campbell WH, Schneider G, Lindqvist Y;

Structure 1994;2:809-821.

385. (oxidored molyb) Eukaryotic molybdopterin oxidoreductases signature

A number of different eukaryotic oxidoreductases that require and bind a molybdopterin cofactor have been shown [1] to share a few regions of sequence similarity. These enzymes are:

- Xanthine dehydrogenase (EC 1.1.1.204), which catalyzes the oxidation of xanthine to uric acid with the concomitant reduction of NAD. Structurally, this enzyme of about 1300 amino acids consists of at least three distinct domains: an N-terminal 2Fe-2S ferredoxin-like iron-sulfur binding domain (see <PDOC00175>), a central FAD/NAD-binding domain and a C-terminal Mo-pterin domain.
- Aldehyde oxidase (EC 1.2.3.1), which catalyzes the oxidation aldehydes into acids. Aldehyde oxidase is highly similar to xanthine dehydrogenase in its sequence and domain structure.
- Nitrate reductase (EC 1.6.6.1), which catalyzes the reduction of nitrate to nitrite. Structurally, this enzyme of about 900 amino acids consists of an N-terminal Mo-pterin domain, a central cytochrome b5-type heme-binding domain (see <PDOC00170>) and a C-terminal FAD/NAD-binding cytochrome reductase domain.
- Sulfite oxidase (EC 1.8.3.1), which catalyzes the oxidation of sulfite to sulfate. Structurally, this enzyme of about 460 amino acids consists of an N-terminal cytochrome b5-binding domain followed by a Mo-pterin domain.

There are a few conserved regions in the sequence of the molybdopterin-binding domain of these enzymes. The pattern used to detect these proteins is based on one of them. It contains a cysteine residue which could be involved in binding the molybdopterin cofactor.

Consensus pattern: [GA]-x(3)-[KRNQHT]-x(11,14)-[LIVMFYWS]-x(8)-[LIVMF]-x-C-x(2)-[DEN]-R-x(2)-[DE]

[1] Wootton J.C., Nicolson R.E., Cock J.M., Walters D.E., Burke J.F., Doyle W.A., Bray R.C. Biochim. Biophys. Acta 1057:157-185(1991).

09555585.061500

386. (Oxidored q1) NADH-Ubiquinone/plastoquinone (complex I), various chains

This family is part of complex I which catalyses the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane. Number of members: 1824

[1]

Medline: 93110040

The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains. Walker JE; Q Rev Biophys 1992;25:253-324.

387. (oxidored q3) NADH-ubiquinone/plastoquinone oxidoreductase chain 6. 179 members.

388. (oxidored q5) NADH-ubiquinone oxidoreductase chain 4, amino terminus

[1] Walker JE ; Q Rev Biophys 1992;25:253-324.

389. (oxidored q6) Respiratory-chain NADH dehydrogenase 20 Kd subunit signature

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 20 Kd (in mammals) [3], which is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind a 4Fe-4S iron-sulfur cluster. The 20 Kd subunit has been found to be:

- Nuclear encoded, as a precursor form with a transit peptide in mammals, and in Neurospora crassa. - Mitochondrial encoded in Paramecium (gene psbG).
- Chloroplast encoded in various higher plants (gene ndhK or psbG).

The 20 Kd subunit is highly similar to [4]:

- Synechocystis strain PCC 6803 proteins psbG1 and psbG2.

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- Subunit B of Escherichia coli NADH-ubiquinone oxidoreductase (gene nuoB).
- Subunit NQO6 of Paracoccus denitrificans NADH-ubiquinone oxidoreductase.
- Subunit 7 of Escherichia coli formate hydrogenlyase (gene hycG).
- Subunit I of Escherichia coli hydrogenase-4 (gene hyfI).

As as signature pattern a highly conserved region was selected, located in the central section of this subunit and which contains a conserved cysteine that is probably involved in the binding of the 4Fe-4S center.

Consensus pattern: [GN]-x-D-[KRST]-[LIVMF](2)-P-[IV]-D-[LIVMFYW](2)-x-P-x-C-P-[PT] [The C is a putative 4Fe-4S ligand]

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).
[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).
[3] Arizmendi J.M., Runswick M.J., Skehel J.M., Walker J.E. FEBS Lett. 301:237-242(1992).
[4] Weidner U., Geier S., Ptock A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-122(1993).

390. p53 tumor antigen signature

The p53 tumor antigen [1 to 5, E1,E2] is a protein found in increased amounts in a wide variety of transformed cells. It is also detectable in many proliferating nontransformed cells, but it is undetectable or present at low levels in resting cells. It is frequently mutated or inactivated in many types of cancer. p53 seems to act as a tumor suppressor in some, but probably not all, tumor types. p53 is probably involved in cell cycle regulation, and may be a trans-activator that acts to negatively regulate cellular division by controlling a set of genes required for this process.

p53 is a phosphoprotein of about 390 amino acids which can be subdivided into four domains: a highly charged acidic region of about 75 to 80 residues, a hydrophobic proline-rich domain (position 80 to 150), a central region (from 150 to about 300), and a highly basic C-terminal region. The sequence of p53 is well conserved in vertebrate species; attempts to identify p53 in other eukaryotic philum has so far been unsuccessful.

As a signature pattern for p53 a perfectly conserved stretch of 13 residues located in the central region of the protein was selected. This region, known as domain IV in [3], is involved (along with an adjacent region) in the binding of the large T antigen of SV40. In man this region is the focus of a variety of point mutations in cancerous tumors.

Consensus pattern: M-C-N-S-S-C-M-G-G-M-N-R-R

[1] Levine A.J., Momand J., Finlay C.A. Nature 351:453-456(1991).
[2] Levine A.J., Momand J. Biochim. Biophys. Acta 1032:119-136(1990).
[3] Soussi T., Caron De Fromentel C., May P. Oncogene 5:945-952(1990).
[4] Lane D.P., Benchimol S. Genes Dev. 4:1-8(1990).
[5] Ulrich S.J., Anderson C.W., Mercer W.E., Appella E. J. Biol. Chem. 267:15259-15262(1992).

09555526.061600

391. (P5CR) Delta 1-pyrroline-5-carboxylate reductase signature

Delta 1-pyrroline-5-carboxylate reductase (P5CR) (EC 1.5.1.2) [1,2] is the enzyme that catalyzes the terminal step in the biosynthesis of proline from glutamate, the NAD(P) dependent oxidation of 1-pyrroline-5-carboxylate into proline.

The sequences of P5CR from eubacteria (gene proC), archaebacteria and eukaryotes show only a moderate level of overall similarity. As a signature pattern, the best conserved region located in the C-terminal section of P5CR was selected.

Consensus pattern: [PALF]-x(2,3)-[LIV]-x(3)-[LIVM]-[STAC]-[STV]-x-[GAN]-G-x-T-x(2)-[AG]-[LIV]-x(2)-[LMF]-[DENQK]

[1] Delauney A.J., Verma D.P. Mol. Gen. Genet. 221:299-305(1990).
[2] Savioz A., Jeenes D.J., Kocher H.P., Haas D. Gene 86:107-111(1990).

392. Poly-adenylate binding protein, unique domain.

393. (PAL) Phenylalanine and histidine ammonia-lyases active site

Phenylalanine ammonia-lyase (EC 4.3.1.5) (PAL) is a key enzyme of plant and fungi phenylpropanoid metabolism which is involved in the biosynthesis of a wide variety of secondary metabolites such as flavanoids, furanocoumarin phytoalexins and cell wall components. These compounds have many important roles in plants during normal growth and in responses to environmental stress. PAL catalyzes the removal of an ammonia group from phenylalanine to form trans-cinnamate.

Histidine ammonia-lyase (EC 4.3.1.3) (histidase) catalyzes the first step in histidine degradation, the removal of an ammonia group from histidine to produce urocanic acid.

The two types of enzymes are functionally and structurally related [1]. They are the only enzymes which are known to have the modified amino acid dehydroalanine (DHA) in their active site. A serine residue has been shown [2,3,4] to be the precursor of this essential electrophilic moiety. The region around this active site residue is well conserved and can be used as a signature pattern.

09595930.041502

Consensus pattern: G-[STG]-[LIVM]-[STG]-[AC]-S-G-[DH]-L-x-P-L-[SA]-x(2)-[SA] [S is the active site residue]

[1] Taylor R.G., Lambert M.A., Sexsmith E., Sadler S.J., Ray P.N., Mahuran D.J., McInnes R.R. J. Biol. Chem. 265:18192-18199(1990).
[2] Langer M., Reck G., Reed J., Retey J. Biochemistry 33:6462-6467(1994).
[3] Schuster B., Retey J. FEBS Lett. 349:252-254(1994).
[4] Taylor R.G., McInnes R.R. J. Biol. Chem. 269:27473-27477(1994).

394. PAS domain

-!- CAUTION. This family does not currently match all known examples of PAS domains.

PAS motifs appear in archaea, eubacteria and eukarya. Probably the most surprising identification of a PAS domain was that in EAG-like K+-channels[1,3].

Number of members: 308

[1]
Medline: 97446881
PAS domain S-boxes in archaea, bacteria and sensors for oxygen and redox.
Zhulin IB, Taylor BL, Dixon R;
Trends Biochem Sci 1997;22:331-333.
[2]Medline: 95275818
1.4 A structure of photoactive yellow protein, a cytosolic photoreceptor: unusual fold, active site, and chromophore.
Borgstahl GE, Williams DR, Getzoff ED;
Biochemistry 1995;34:6278-6287.
[3]Medline: 98044337
PAS: a multifunctional domain family comes to light.
Ponting CP, Aravind L;
Curr Biol 1997;7:674-677.

09505305.051600

395. (PBP) Phosphatidylethanolamine-binding protein family signature

Mammalian phosphatidylethanolamine-binding protein (also knowns as basic cytosolic 21 Kd protein) is a 186 residue protein found in a variety of tissues [1]. It binds hydrophobic ligands, such as phosphatidylethanolamine, but also seems [2] to bind nucleotides such as GTP and FMN, it is suggested that it could act in membrane remodeling during growth and maturation. This protein belongs to a family that also includes:

- Drosophila antennal protein A5, a putative odorant-binding protein.
- Onchocerca volvulus antigen Ov-16 and the related proteins D1, D2 and D3.
- Plasmodium falciparum putative phosphatidylethanolamine-binding protein.
- Toxocara canis secreted antigen TES-26. This larval protein has been shown to bind phosphatidylethanolamine.

- Yeast protein DKA1 (also known as NSP1 or TFS1). The function of this protein is not very clear. - Yeast hypothetical protein YLR179C.
- Caenorhabditis elegans hypothetical protein F40A3.3.

As a signature pattern, the best conserved region was selected which is located in the end of the first third of the sequence of these proteins.

Consensus pattern: [FYL]-x-[LV]-[LIVF]-x-[TIV]-[DC]-P-D-x-P-[SN]-x(10)-H

[1] Seddiqi N., Bollengier F., Alliel P.M., Perin J.P., Bonnet F., Bucquoy S., Jolles P., Schoentgen F. J. Mol. Evol. 39:655-660(1994).
[2] Schoentgen F., Jolles P. FEBS Lett. 369:22-6(1995).

09554573.051900

396. PCI domain

This domain has also been called the PINT motif (Proteasome, Int-6, Nip-1 and TRIP-15) [1].

Number of members: 49

[1]

Medline: 98308842

The PCI domain: a common theme in three multiprotein complexes.

Hofmann K, Bucher P;

Trends Biochem Sci 1998;23:204-205.

[2]Medline: 98266368

Homologues of 26S proteasome subunits are regulators of transcription and translation.

Aravind L, Ponting CP;

Protein Sci 1998;7:1250-1254.

397. (PCMT) Protein-L-isoaspartate (D-aspartate) O-methyltransferase signature. Protein-L-isoaspartate (D-aspartate) O-methyltransferase (EC 2.1.1.77) (PCMT)[1] (which is also known as L-isoaspartyl protein carboxyl methyltransferase)is an enzyme that catalyzes the

transfer of a methyl group from S-adenosylmethionine to the free carboxyl groups of D-aspartyl or L-isoaspartyl residues in a variety of peptides and proteins. The enzyme does not act on normal L-aspartyl residues L-isoaspartyl and D-aspartyl are the products of the spontaneous de amidation and/or isomerization of normal L-aspartyl and L-asparaginyl residues in proteins. PCMT plays a role in the repair and/ordegradation of these damaged proteins; the enzymatic methyl esterification of the abnormal residues can lead to their conversion to normal L-aspartylresidues. PCMT is a well-conserved and widely distributed cytosolic protein of about 24Kd. As a signature pattern, a conserved region in the central part of this enzyme has been developed.

Consensus pattern: [GSA]-D-G-x(2)-G-[FYWV]-x(3)-[AS]-P-[FY]-[DN]-x-I –

[1] Kagan R.M., McFadden H.J., McFadden P.N., O'Connor C., Clarke S. Comp. Biochem. Physiol. 117b:379-385(1997).

09555356.061502

398. (PCNA) Proliferating cell nuclear antigen signatures

Proliferating cell nuclear antigen (PCNA) [1,2] is a protein involved in DNA replication by acting as a cofactor for DNA polymerase delta, the polymerase responsible for leading strand DNA replication.

A similar protein exists in yeast (gene POL30) [3] and is associated with polymerase III, the yeast analog of polymerase delta. In baculoviruses the ETL protein has been shown [4] to be highly related to PCNA and is probably associated with the viral encoded DNA polymerase. An homolog of PCNA is also found in archebacteria.

As signatures for this family of proteins, two conserved regions were selected located in the N-terminal section. The second one has been proposed to bind DNA.

Consensus pattern: [GA]-[LIVMF]-x-[LIVMA]-x-[SAV]-[LIVM]-D-x-[NSAE]-[HKR]-[VI]-x-[LY]-[VGA]-x-[LIVM]-x-[LIVM]-x(4)-F

-Consensus pattern: [RKA]-C-[DE]-[RH]-x(3)-[LIVMF]-x(3)-[LIVM]-x-[SGAN]-[LIVMF]-x-K-[LIVMF](2)

[1] Bravo R., Frank R., Blundell P.A., McDonald-Bravo H. Nature 326:515-517(1987).
[2] Suzuka I., Hata S., Matsuoka M., Kosugi S., Hashimoto J. Eur. J. Biochem. 195:571-575(1991).[3] Bauer G.A., Burgess P.M.J. Nucleic Acids Res. 18:261-265(1990).
[4] O'Reilly D.R., Crawford A.M., Miller L.K. Nature 337:606-606(1989).

399. (PDT) Prephenate dehydratase signatures

Prephenate dehydratase (EC 4.2.1.51) (PDT) catalyzes the decarboxylation of prephenate into phenylpyruvate. In microorganisms PDT is involved in the terminal pathway of the biosynthesis of phenylalanine. In some bacteria such as Escherichia coli PDT is part of a bifunctional enzyme (P-protein) that also catalyzes the transformation of chorismate into prephenate (chorismate mutase) while in other bacteria it is a monofunctional enzyme. The sequence of monofunctional PDT align well with the C-terminal part of that of P-proteins [1].

As signature patterns for PDT two conserved regions were selected. The first region contains a conserved threonine which has been said to be essential for the activity of the enzyme in E. coli. The second region includes a conserved glutamate. Both regions are in the C-terminal part of PDT.

Consensus pattern: [FY]-x-[LIVM]-x(2)-[LIVM]-x(5)-[DN]-x(5)-T-R-F-[LIVMW]-x-[LIVM]

[1] Fischer R.S., Zhao G., Jensen R.A. J. Gen. Microbiol. 137:1293-1301(1991).

400. PDZ domain (Also known as DHR or GLGF).

PDZ domains are found in diverse signaling proteins.

[1] Ponting CP, Phillips C, Davies KE, Blake DJ Bioessays 1997;19:469-479. [2] Doyle DA, Lee A, Lewis J, Kim E, Sheng M, MacKinnon R; Cell. 1996;85:1067-1076. [3] Ponting CP; Protein Sci 1997;6:464-468.

401. (PPDK_N_term) PEP-utilizing enzymes signatures

A number of enzymes that catalyze the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) via a phospho-histidine intermediate have been shown to be structurally related [1,2,3,4]. These enzymes are:

- Pyruvate,orthophosphate dikinase (EC 2.7.9.1) (PPDK). PPDK catalyzes the reversible phosphorylation of pyruvate and phosphate by ATP to PEP and diphosphate. In plants PPDK function in the direction of the formation of PEP, which is the primary acceptor of carbon dioxide in C4 and crassulacean acid metabolism plants. In some bacteria, such as Bacteroides symbiosus, PPDK functions in the direction of ATP synthesis.
- Phosphoenolpyruvate synthase (EC 2.7.9.2) (pyruvate,water dikinase). This enzyme catalyzes the reversible phosphorylation of pyruvate by ATP to form PEP, AMP and phosphate, an essential step in gluconeogenesis when pyruvate and lactate are used as a carbon source.
- Phosphoenolpyruvate-protein phosphotransferase (EC 2.7.3.9). This is the first enzyme of the phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS), a major carbohydrate transport system in bacteria. The PTS catalyzes the phosphorylation of incoming sugar substrates concomitant with their translocation across the cell membrane. The general mechanism of the PTS is the following: a phosphoryl group from PEP is transferred to enzyme-I (EI) of PTS which in turn transfers it to a phosphoryl carrier protein (HPr). Phospho-HPr then transfers the phosphoryl group to a sugar-specific permease.

All these enzymes share the same catalytic mechanism: they bind PEP and transfer the phosphoryl group from it to a histidine residue. The sequence around that residue is highly conserved and can be used as a signature pattern for these enzymes. As a second signature pattern a conserved region was selected in the C-terminal part of the PEP-utilizing enzymes. The biological significance of this region is not yet known.

Consensus pattern: G-[GA]-x-[TN]-x-H-[STA]-[STAV]-[LIVM](2)-[STAV]-[RG] [H is phosphorylated]

-Consensus pattern: [DEQSK]-x-[LIVMF]-S-[LIVMF]-G-[ST]-N-D-[LIVM]-x-Q-[LIVMFYGT]-[STALIV]-[LIVMF]-[GAS]-x(2)-R

[1] Reizer J., Hoischen C., Reizer A., Pham T.N., Saier M.H. Jr. Protein Sci. 2:506-521(1993).
[2] Reizer J., Reizer A., Merrick M.J., Plunkett G. III, Rose D.J., Saier M.H. Jr. Gene 181:103-108(1996).
[3] Pocalyko D.J., Carroll L.J., Martin B.M., Babbitt P.C., Dunaway-Mariano D. Biochemistry 29:10757-10765(1990).
[4] Niersbach M., Kreuzaler F., Geerse R.H., Postma P., Hirsch H.J. Mol. Gen. Genet. 232:332-336(1992).

402. (PEPCK ATP) Phosphoenolpyruvate carboxykinase (ATP) signature

Phosphoenolpyruvate carboxykinase (ATP) (EC 4.1.1.49) (PEPCK) [1] catalyzes the formation of phosphoenolpyruvate by decarboxylation of oxaloacetate while hydrolyzing ATP, a rate limiting step in gluconeogenesis (the biosynthesis of glucose).

The sequence of this enzyme has been obtained from Escherichia coli, yeast, and Trypanosoma brucei; these three sequences are evolutionary related and share many regions of similarity. As a signature pattern a highly conserved region was selected that contains four acidic residues and which is located in the central part of the enzyme. The beginning of the pattern is located about 10 residues to the C-terminus of an ATP-binding motif 'A' (P-loop) (see <PDOC00017>) and is also part of the ATP-binding domain [2].

Consensus pattern: L-I-G-D-D-E-H-x-W-x-[DE]-x-G-[IV]-x-N

-Note: phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) an enzyme that catalyzes the same reaction, but using GTP instead of ATP, is not related to the above enzyme (see <PDOC00421>).

[1] Medina V., Pontarollo R., Glaeske D., Tabel H., Goldie H. J. Bacteriol. 172:7151-7156(1990).

[2] Matte A., Goldie H., Sweet R.M., Delbaere L.T.J. J. Mol. Biol. 256:126-143(1996).

403. (Pepcase) Phosphoenolpyruvate carboxylase active sites. Phosphoenolpyruvate carboxylase (EC 4.1.1.31) (PEPcase) catalyzes the irreversible beta-carboxylation of phosphoenolpyruvate by bicarbonate to yield oxaloacetate and phosphate. The enzyme is found in all plants and in a variety of microorganisms. A histidine [1] and a lysine [2] have been implicated in the catalytic mechanism of this enzyme; the regions around these active site residues are highly conserved in PEPcase from various plants, bacteria and cyanobacteria and can be used as a signature patterns for this type of enzyme.

Consensus pattern: [VT]-x-T-A-H-P-T-[EQ]-x(2)-R-[KRH] [H is an active site residue]-
Consensus pattern: [IV]-M-[LIVM]-G-Y-S-D-S-x-K-D-[STAG]-G [K is an active site residue]-

[1] Terada K., Izui K. Eur. J. Biochem. 202:797-803(1991).[2] Jiao J.-A., Podesta F.E., Chollet R., O'Leary M.H., Andreo C.S. Biochim. Biophys. Acta 1041:291-295(1990).

404. PET112 family signature

The following proteins from eukaryotes, prokaryotes and archaebacteria belong to the same family:

- Yeast mitochondrial protein PET112 [1], which plays an unknown role in the expression of mitochondrial genes, probably at the level of translation.
- Aspergillus nidulans mitochondrial protein nempA.
- Bacillus subtilis hypothetical protein yzdD.
- Moraxella catarrhalis hypothetical protein in bloR-1 3'region.
- Mycoplasma genitalium hypothetical protein MG100.
- Methanococcus jannaschii hypothetical proteins MJ0019 and MJ0160.

The size of these proteins range from 419 to 630 amino acids. As a signature pattern, a conserved region located in the N-terminal section was selected.

Consensus pattern: [DN]-x-[DN]-R-x(3)-P-L-[LIV]-E-[LIV]-x-[ST]-x-P

[1] Mulero J.J., Rosenthal J.K., Fox T.D. Curr. Genet. 25:299-304(1994).

405. (PFK) Phosphofructokinase signature

Phosphofructokinase (EC 2.7.1.11) (PFK) [1,2] is a key regulatory enzyme in the glycolytic pathway. It catalyzes the phosphorylation by ATP of fructose 6-phosphate to fructose 1,6-bisphosphate. In bacteria PFK is a tetramer of identical 36 Kd subunits. In mammals it is a tetramer of 80 Kd subunits. Each 80 Kd subunit consist of two homologous domains which are highly related to the bacterial 36 Kd subunits. In Human there are three, tissue-specific, types of PFK isozymes: PFKM (muscle), PFKL (liver), and PFKP (platelet). In yeast PFK is an octamer composed of four 100 Kd alpha chains (gene PFK1) and four 100 Kd beta chains (gene PFK2); like the mammalian 80 Kd subunits, the yeast 100 Kd subunits are composed of two homologous domains.

As a signature pattern for PFK a region that contains three basic residues involved in fructose-6-phosphate binding was selected.

Consensus pattern: [RK]-x(4)-G-H-x-Q-[QR]-G-G-x(5)-D-R [The R/K, the H and the Q/R are involved in fructose-6-P binding]

-Note: Escherichia coli has two phosphofructokinase isozymes which are encoded by genes pfkA (major) and pfkB (minor). The pfkB isozyme is not evolutionary related to other prokaryotic or eukaryotic PFK's (see <PDOC00504>).

[1] Poorman R.A., Randolph A., Kemp R.G., Heinrikson R.L. Nature 309:467-469(1984).
[2] Heinisch J., Ritzel R.G., von Borstel R.C., Aguilera A., Rodicio R., Zimmermann F.K. Gene 78:309-321(1989).

406. (PGAM) Phosphoglycerate mutase family phosphohistidine signature

Phosphoglycerate mutase (EC 5.4.2.1) (PGAM) and bisphosphoglycerate mutase (EC 5.4.2.4) (BPGM) are structurally related enzymes which catalyze reactions involving the transfer of phospho groups between the three carbon atoms of phosphoglycerate [1,2]. Both enzymes can catalyze three different reactions, although in different proportions:

- The isomerization of 2-phosphoglycerate (2-PGA) to 3-phosphoglycerate (3-PGA) with 2,3-diphosphoglycerate (2,3-DPG) as the primer of the reaction.
- The synthesis of 2,3-DPG from 1,3-DPG with 3-PGA as a primer.
- The degradation of 2,3-DPG to 3-PGA (phosphatase EC 3.1.3.13 activity).

In mammals, PGAM is a dimeric protein. There are two isoforms of PGAM: the M (muscle) and B (brain) forms. In yeast, PGAM is a tetrameric protein. BPGM is a dimeric protein and is found mainly in erythrocytes where it plays a major role in regulating hemoglobin oxygen affinity as a consequence of controlling 2,3-DPG concentration.

The catalytic mechanism of both PGAM and BPGM involves the formation of a phosphohistidine intermediate [3].

The bifunctional enzyme 6-phosphofructo-2-kinase / fructose-2,6-bisphosphatase (EC 2.7.1.105 and EC 3.1.3.46) (PF2K) [4] catalyzes both the synthesis and the degradation of fructose-2,6-bisphosphate. PF2K is an important enzyme in the regulation of hepatic carbohydrate metabolism. Like PGAM/BPGM, the fructose-2,6-bisphosphatase reaction involves a phosphohistidine intermediate and the phosphatase domain of PF2K is structurally related to PGAM/BPGM.

The bacterial enzyme alpha-ribazole-5'-phosphate phosphatase (gene cobC) which is involved in cobalamin biosynthesis also belongs to this family [5].

A signature pattern was built around the phosphohistidine residue.

Consensus pattern: [LIVM]-x-R-H-G-[EQ]-x(3)-N [H is the phosphohistidine residue]

-Note: some organisms harbor a form of PGAM independent of 2,3-DPG, this enzyme is not related to the family described above [6].

[1] Le Boulch P., Joulin V., Garel M.-C., Rosa J., Cohen-Solal M. Biochem. Biophys. Res. Commun. 156:874-881(1988).

[2] White M.F., Fothergill-Gilmore L.A. FEBS Lett. 229:383-387(1988).

[3] Rose Z.B. Meth. Enzymol. 87:43-51(1982).

[4] Bazan J.F., Fletterick R.J., Pilkis S.J. Proc. Natl. Acad. Sci. U.S.A. 86:9642-9646(1989).

[5] O'Toole G.A., Trzebiatowski J.R., Escalante-Semerena J.C. J. Biol. Chem. 269:26503-26511(1994).

[6] Grana X., De Lecea L., El-Maghrabi M.R., Urena J.M., Caellas C., Carreras J., Puigdomenech P., Pilkis S.J., Climent F. J. Biol. Chem. 267:12797-12803(1992).

407. (PGI) Phosphoglucose isomerase signatures

Phosphoglucose isomerase (EC 5.3.1.9) (PGI) [1,2] is a dimeric enzyme that catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate. PGI is involved in different pathways: in most higher organisms it is involved in glycolysis; in mammals it is involved in gluconeogenesis; in plants in carbohydrate biosynthesis; in some bacteria it provides a gateway for fructose into the Entner-Doudouroff pathway. PGI has been shown [3] to be identical to neuroleukin, a neurotrophic factor which supports the survival of various types of neurons.

The sequence of PGI from many species ranging from bacteria to mammals is available and has been shown to be highly conserved. As signature patterns for this enzyme two conserved regions were selected, the first region is located in the central section of PGI, while the second one is located in its C-terminal section.

Consensus pattern: [DENS]-x-[LIVM]-G-G-R-[FY]-S-[LIVMT]-x-[STA]-[PSAC]-[LIVMA]-G

-Consensus pattern: [GS]-x-[LIVM]-[LIVMFYW]-x(4)-[FY]-[DN]-Q-x-G-V-E-x(2)-K

[1] Achari A., Marshall S.E., Muirhewad H., Palmieri R.H., Noltmann E.A. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 293:145-157(1981).

[2] Smith M.W., Doolittle R.F. J. Mol. Evol. 34:544-545(1992).

[3] Faik P., Walker J.I.H., Redmill A.A.M., Morgan M.J. Nature 332:455-456(1988).

408. (PGK) Phosphoglycerate kinase signature

Phosphoglycerate kinase (EC 2.7.2.3) (PGK) [1] catalyzes the second step in the second phase of glycolysis, the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate with generation of one molecule of ATP. PGK is found in all living organisms and its sequence has been highly conserved throughout evolution. It is a two-domain protein; each domain is composed of six repeats of an alpha/beta structural motif. As a signature pattern for PGK's, a conserved region in the N-terminal region was selected.

Consensus pattern: [KRHGTCVN]-[VT]-[LIVMF]-[LIVMC]-R-x-D-x-N-[SACV]-P

[1] Watson H.C., Littlechild J.A. Biochem. Soc. Trans. 18:187-190(1990).

09595326.052502

409. (PGM PMM) Phosphoglucomutase and phosphomannomutase phosphoserine signature

- Phosphoglucomutase (EC 5.4.2.2) (PGM). PGM is an enzyme responsible for the conversion of D-glucose 1-phosphate into D-glucose 6-phosphate. PGM participates in both the breakdown and synthesis of glucose [1].
- Phosphomannomutase (EC 5.4.2.8) (PMM). PMM is an enzyme responsible for the conversion of D-mannose 1-phosphate into D-mannose 6-phosphate. PMM is required for different biosynthetic pathways in bacteria. For example, in enterobacteria such as Escherichia coli there are two different genes coding for this enzyme: rfbK which is involved in the synthesis of the O antigen of lipopolysaccharide and cpsG which is required for the synthesis of the M antigen capsular polysaccharide [2]. In Pseudomonas aeruginosa PMM (gene algC) is involved in the biosynthesis of the alginate layer [3] and in Xanthomonas campestris (gene xanA) it is involved in the biosynthesis of xanthan [4]. In Rhizobium strain ngr234 (gene noeK) it is involved in the biosynthesis of the nod factor.
- Phosphoacetylglucosamine mutase (EC 5.4.2.3) which converts N-acetyl-D-glucosamine 1-phosphate into the 6-phosphate isomer.

The catalytic mechanism of both PGM and PMM involves the formation of a phosphoserine intermediate [1]. The sequence around the serine residue is well conserved and can be used as a signature pattern.

In addition to PGM and PMM there are at least three uncharacterized proteins that belong to this family [5,6]:

- Urease operon protein ureC from Helicobacter pylori.
- Escherichia coli protein mrsA.
- Paramecium tetraurelia parafusin, a phosphoglycoprotein involved in exocytosis.
- A Methanococcus vannielii hypothetical protein in the 3'region of the gene for ribosomal protein S10.

Consensus pattern: [GSA]-[LIVM]-x-[LIVM]-[ST]-[PGA]-S-H-x-P-x(4)-[GNHE] [S is the phosphoserine residue]

-Note: PMM from fungi do not belong to this family.

[1] Dai J.B., Liu Y., Ray W.J. Jr., Konno M. J. Biol. Chem. 267:6322-6337(1992).

[2] Stevenson G., Lee S.J., Romana L.K., Reeves P.R. Mol. Gen. Genet. 227:173-180(1991).

[3] Zielinski N.A., Chakrabarty A.M., Berry A. J. Biol. Chem. 266:9754-9763(1991).

[4] Koeplin R., Arnold W., Hoette B., Simon R., Wang G., Puehler A. J. Bacteriol. 174:191-199(1992).

[5] Bairoch A. Unpublished observations (1993).

[6] Subramanian S.V., Wyroba E., Andersen A.P., Satir B.H. Proc. Natl. Acad. Sci. U.S.A. 91:9832-9836(1994).

410. PH domain profile

The 'pleckstrin homology' (PH) domain is a domain of about 100 residues that occurs in a wide range of proteins involved in intracellular signaling or as constituents of the cytoskeleton [1 to 7].

The function of this domain is not clear, several putative functions have been suggested: - binding to the beta/gamma subunit of heterotrimeric G proteins,

- binding to lipids, e.g. phosphatidylinositol-4,5-bisphosphate,
- binding to phosphorylated Ser/Thr residues,
- attachment to membranes by an unknown mechanism.

It is possible that different PH domains have totally different ligand requirements.

The 3D structure of several PH domains has been determined [8]. All known cases have a common structure consisting of two perpendicular anti-parallel beta sheets, followed by a C-terminal amphipathic helix. The loops connecting the beta-strands differ greatly in length, making the PH domain relatively difficult to detect. There are no totally invariant residues within the PH domain.

Proteins reported to contain one more PH domains belong to the following families:

- Pleckstrin, the protein where this domain was first detected, is the major substrate of protein kinase C in platelets. Pleckstrin is one of the rare proteins to contains two PH domains.
- Ser/Thr protein kinases such as the Act/Rac family, the beta-adrenergic receptor kinases, the mu isoform of PKC and the trypanosomal NrkA family.
- Tyrosine protein kinases belonging to the Btk/Itk/Tec subfamily.
- Insulin Receptor Substrate 1 (IRS-1).
- Regulators of small G-proteins like guanine nucleotide releasing factor GNRP (Ras-GRF) (which contains 2 PH domains), guanine nucleotide exchange proteins like vav, dbl, SoS and yeast CDC24, GTPase activating proteins like rasGAP and BEM2/IPL2, and the human break point cluster protein bcr.
- Cytoskeletal proteins such as dynamin (see <PDOC00362>), Caenorhabditis elegans kinesin-like protein unc-104 (see <PDOC00343>), spectrin beta-chain, syntrophin (2 PH domains) and yeast nuclear migration protein NUM1.
- Mammalian phosphatidylinositol-specific phospholipase C (PI-PLC) (see <PDOC50007>) isoforms gamma and delta. Isoform gamma contains two PH domains, the second one is split into two parts separated by about 400 residues. - Oxysterol binding proteins OSBP, yeast OSH1 and YHR073w.
- Mouse protein citron, a putative rho/rac effector that binds to the GTP-bound forms of rho and rac,
- Several yeast proteins involved in cell cycle regulation and bud formation like BEM2, BEM3, BUD4 and the BEM1-binding proteins BOI2 (BEB1) and BOI1 (BOB1). - Caenorhabditis elegans protein MIG-10.
- Caenorhabditis elegans hypothetical proteins C04D8.1, K06H7.4 and ZK632.12.
- Yeast hypothetical proteins YBR129c and YHR155w.

The profile for the PH domain, which has been developed by Toby Gibson at the EMBL, covers the total length of domain. Several proteins contain large insertions in the PH domain and are thus difficult to detect with this profile. In some of these cases, the profile will align only to one half of the PH domain.

-Sequences known to belong to this class detected by the pattern: ALL. But it should be noted that while all sequences containing PH domains are detected, not all PH domains are. Some of the split domains lie below the cutoff threshold.

[1] Mayer B.J., Ren R., Clark K.L., Baltimore D. Cell 73:629-630(1993).
[2] Haslam R.J., Koide H.B., Hemmings B.A. Nature 363:309-310(1993).
[3] Musacchio A., Gibson T.J., Rice P., Thompson J., Saraste M. Trends Biochem. Sci. 18:343-348(1993).
[4] Gibson T.J., Hyvonen M., Musacchio A., Saraste M., Birney E. Trends Biochem. Sci. 19:349-353(1994).[5] Pawson T. Nature 373:573-580(1995).[6] Ingley E., Hemmings B.A. J. Cell. Biochem. 56:436-443(1994).[7] Saraste M., Hyvonen M. Curr. Opin. Struct. Biol. 5:403-408(1995).[8] Riddihough G. Nat. Struct. Biol. 1:755-757(1994).

411. PHD-finger

[1]

Medline: 95216093

The PHD finger: implications for chromatin-mediated transcriptional regulation.

Aasland R, Gibson TJ, Stewart AF;

Trends Biochem Sci 1995;20:56-59.

Number of members: 181

412. (PI-PLC-X) Phosphatidylinositol-specific phospholipase C profiles

Phosphatidylinositol-specific phospholipase C (EC 3.1.4.11), an eukaryotic intracellular enzyme, plays an important role in signal transduction processes [1]. It catalyzes the hydrolysis of 1-phosphatidyl-D-myo-inositol-3,4,5-triphosphate into the second messenger molecules diacylglycerol and inositol-1,4,5-triphosphate. This catalytic process is tightly regulated by reversible phosphorylation and binding of regulatory proteins [2 to 4].

In mammals, there are at least 6 different isoforms of PI-PLC, they differ in their domain structure, their regulation, and their tissue distribution. Lower eukaryotes also possess multiple isoforms of PI-PLC.

All eukaryotic PI-PLCs contain two regions of homology, sometimes referred to as 'X-box' and 'Y-box'. The order of these two regions is always the same (NH2-X-Y-COOH), but the spacing is variable. In most isoforms, the distance between these two regions is only 50-100 residues but in the gamma isoforms one PH domain, two SH2 domains, and one SH3 domain are inserted between the two PLC-specific domains. The two conserved regions have been shown to be important for the catalytic activity. At the C-terminal of the Y-box, there is a C2 domain (see <PDOC00380>) possibly involved in Ca-dependent membrane attachment.

Profile analysis shows that sequences with significant similarity to the X-box domain occur also in prokaryotic and trypanosome PI-specific phospholipases C. Apart from this region, the prokaryotic enzymes show no similarity to their eukaryotic counterparts.

Two profiles were developed, one covering the X-box, the other the Y-box.

[1] Meldrum E., Parker P.J., Carozzi A.
Biochim. Biophys. Acta 1092:49-71(1991).[2] Rhee S.G., Choi K.D.
Adv. Second Messenger Phosphoprotein Res. 26:35-61(1992).
[3] Rhee S.G., Choi K.D. J. Biol. Chem. 267:12393-12396(1992).
[4] Sternweis P.C., Smrcka A.V. Trends Biochem. Sci. 17:502-506(1992).

413. (PI-PLC-Y) Phosphatidylinositol-specific phospholipase C profiles

Phosphatidylinositol-specific phospholipase C (EC 3.1.4.11), an eukaryotic intracellular enzyme, plays an important role in signal transduction processes [1]. It catalyzes the hydrolysis of 1-phosphatidyl-D-myo-inositol-3,4,5-

09525325.061501

triphosphate into the second messenger molecules diacylglycerol and inositol-1,4,5-triphosphate. This catalytic process is tightly regulated by reversible phosphorylation and binding of regulatory proteins [2 to 4].

In mammals, there are at least 6 different isoforms of PI-PLC, they differ in their domain structure, their regulation, and their tissue distribution. Lower eukaryotes also possess multiple isoforms of PI-PLC.

All eukaryotic PI-PLCs contain two regions of homology, sometimes referred to as 'X-box' and 'Y-box'. The order of these two regions is always the same (NH2-X-Y-COOH), but the spacing is variable. In most isoforms, the distance between these two regions is only 50-100 residues but in the gamma isoforms one PH domain, two SH2 domains, and one SH3 domain are inserted between the two PLC-specific domains. The two conserved regions have been shown to be important for the catalytic activity. At the C-terminal of the Y-box, there is a C2 domain (see <PDOC00380>) possibly involved in Ca-dependent membrane attachment.

Profile analysis shows that sequences with significant similarity to the X-box domain occur also in prokaryotic and trypanosome PI-specific phospholipases C. Apart from this region, the prokaryotic enzymes show no similarity to their eukaryotic counterparts.

Two profiles were developed, one covering the X-box, the other the Y-box.

[1] Meldrum E., Parker P.J., Carozzi A.
Biochim. Biophys. Acta 1092:49-71(1991).[2] Rhee S.G., Choi K.D.
Adv. Second Messenger Phosphoprotein Res. 26:35-61(1992).
[3] Rhee S.G., Choi K.D. J. Biol. Chem. 267:12393-12396(1992).
[4] Sternweis P.C., Smrcka A.V. Trends Biochem. Sci. 17:502-506(1992).

414. (PK) Pyruvate kinase active site signature

Pyruvate kinase (EC 2.7.1.40) (PK) [1] catalyzes the final step in glycolysis, the conversion of phosphoenolpyruvate to pyruvate with the concomitant phosphorylation of ADP to ATP. PK requires both magnesium and potassium ions for its activity. PK is found in all living organisms. In vertebrates there are four, tissues specific, isozymes: L (liver), R (red cells), M1 (muscle, heart, and brain), and M2 (early fetal tissues). In Escherichia coli there are two isozymes: PK-I (gene pykF) and PK-II (gene pykA). All PK isozymes seem to be tetramers of identical subunits of about 500 amino acid residues.

As a signature pattern for PK a conserved region was selected that includes a lysine residue which seems to be the acid/base catalyst responsible for the interconversion of pyruvate and enolpyruvate, and a glutamic acid residue implicated in the binding of the magnesium ion.

Consensus pattern: [LIVAC]-x-[LIVM](2)-[SAPCV]-K-[LIV]-E-[NKRST]-x-[DEQHS]-[GSTA]-[LIVM] [K is the active site residue] [E is a magnesium ligand]

[1] Muirhead H. Biochem. Soc. Trans. 18:193-196(1990).

09555326.051600

415. (PLDc) Phospholipase D. Active site motif

Phosphatidylcholine-hydrolyzing phospholipase D (PLD) isoforms are activated by ADP-ribosylation factors (ARFs). PLD produces phosphatidic acid from phosphatidylcholine, which may be essential for the formation of certain types of transport vesicles or may be constitutive vesicular transport to signal transduction pathways.

PC-hydrolyzing PLD is a homologue of cardiolipin synthase, phosphatidylserine synthase, bacterial PLDs, and viral proteins.

Each of these appears to possess a domain duplication which is apparent by the presence of two motifs containing well-conserved histidine, lysine, and/or asparagine residues which may contribute to the active site. aspartic acid. An E. coli endonuclease (nuc) and similar proteins appear to be PLD homologues but possess only one of these motifs.

The profile contained here represents only the putative active site regions, since an accurate multiple alignment of the repeat units has not been achieved.

Number of members: 139

[1]

Medline: 96303814

A novel family of phospholipase D homologues that includes
phospholipid synthases and putative endonucleases:
identification of duplicated repeats and potential active
site residues.
Ponting CP, Kerr ID;
Protein Sci 1996;5:914-922.
[2]Medline: 96334293
A duplicated catalytic motif in a new superfamily of
phosphohydrolases and phospholipid synthases that includes
poxvirus envelope proteins.
Koonin EV;
Trends Biochem Sci 1996;21:242-243.
[3]Medline: 94327597
Cloning and expression of phosphatidylcholine-hydrolyzing
phospholipase D from Ricinus communis L.
Wang X, Xu L, Zheng L;
J Biol Chem 1994;269:20312-20317.
[4]Medline: 97386825
Regulation of eukaryotic phosphatidylinositol-specific
phospholipase C and phospholipase D.
Singer WD, Brown HA, Sternweis PC;
Annu Rev Biochem 1997;66:475-509.

09553650.031700

416. (PMI type1) Phosphomannose isomerase type I signatures

Phosphomannose isomerase (EC 5.3.1.8) (PMI) [1,2] is the enzyme that catalyzes the interconversion of mannose-6-phosphate and fructose-6-phosphate. In eukaryotes, it is involved in the synthesis of GDP-mannose which is a constituent of N- and O-linked glycans as well as GPI anchors. In prokaryotes, it is involved in a variety of pathways including capsular polysaccharide biosynthesis and D-mannose metabolism.

Three classes of PMI have been defined on the basis of sequence similarities [1]. The first class comprises all known eukaryotic PMI as well as the enzyme encoded by the manA gene in enterobacteria such as Escherichia coli. Class I PMI's are proteins of about 42 to 50 Kd which bind a zinc ion essential for their activity.

As signature patterns for class I PMI, two conserved regions were selected. The first one is located in the N-terminal section of these proteins, the second in the C-terminal half. Both patterns contain a residue involved [3] in the binding of the zinc ion.

Consensus pattern: Y-x-D-x-N-H-K-P-E [E is a zinc ligand]

-Consensus pattern: H-A-Y-[LIVM]-x-G-x(2)-[LIVM]-E-x-M-A-x-S-D-N-x-[LIVM]-R-A-G-x-T-P-K [H is a zinc ligand]

09535552.051400

[1] Proudfoot A.E.I., Turcatti G., Wells T.N.C., Payton M.A., Smith D.J. Eur. J. Biochem. 219:415-423(1994).

[2] Coulin F., Magnenat E., Proudfoot A.E.I., Payton M.A., Scully P., Wells T.N.C. Biochemistry 32:14139-14144(1993).

[3] Cleasby A., Wonacott A., Skarzynski T., Hubbard R.E., Davies G.J., Proudfoot A.E.I., Bernard A.R., Payton M.A., Wells T.N.C. Nat. Struct. Biol. 3:470-479(1996).

417. (PNP UDP 1) Purine and other phosphorylases family 1 signature

The following phosphorylases belongs to the same family:

- Purine nucleoside phosphorylase (EC 2.4.2.1) (PNP) from most bacteria (gene deoD). This enzyme catalyzes the cleavage of guanosine or inosine to respective bases and sugar-1-phosphate molecules [1].
- Uridine phosphorylase (EC 2.4.2.3) (UdRPase) from bacteria (gene udp) and mammals. Catalyzes the cleavage of uridine into uracil and ribose-1-phosphate. The products of the reaction are used either as carbon and energy sources or in the rescue of pyrimidine bases for nucleotide synthesis [2].
- 5'-methylthioadenosine phosphorylase (EC 2.4.2.28) (MTA phosphorylase) from Sulfolobus solfataricus [3].

As a signature pattern, a conserved region was selected in the central part of these enzymes.

Consensus pattern: [GST]-x-G-[LIVM]-G-x-[PA]-S-x-[GSTA]-I-x(3)-E-L

-Note: it shoudl be noted that mammalian and some bacterial PNP as well as eukaryotic MTA phosphorylase belong to a different family of phosphorylases (see <PDOC00954>).

[1] Takehara M., Ling F., Izawa S., Inoue Y., Kimura A. Biosci. Biotechnol. Biochem. 59:1987-1990(1995).

[2] Watanabe S.-I., Hino A., Wada K., Eliason J.F., Uchida T. J. Biol. Chem. 270:12191-12196(1995).

[3] Cacciapuoti G., Porcelli M., Bertoldo C., De Rosa M., Zappia V. J. Biol. Chem. 269:24762-24769(1994).

09555206 . 061901

418. (PP2C) Protein phosphatase 2C signature

Protein phosphatase 2C (PP2C) is one of the four major classes of mammalian serine/threonine specific protein phosphatases (EC 3.1.3.16). PP2C [1] is a monomeric enzyme of about 42 Kd which shows broad substrate specificity and is dependent on divalent cations (mainly manganese and magnesium) for its activity. Its exact physiological role is still unclear. Three isozymes are currently known in mammals: PP2C-alpha, -beta and -gamma. In yeast, there are at least four PP2C homologs: phosphatase PTC1 [2] which has weak tyrosine phosphatase activity in addition to its activity on serines, phosphatases PTC2 and PTC3, and hypothetical protein YBR125c. Isozymes of PP2C are also known from Arabidopsis thaliana (ABI1, PPH1), Caenorhabditis elegans (FEM-2, F42G9.1, T23F11.1), Leishmania chagasi and Paramecium tetraurelia.

In Arabidopsis thaliana, the kinase associated protein phosphatase (KAPP) [3] is an enzyme that dephosphorylates the Ser/Thr receptor-like kinase RLK5 and which contains a C-terminal PP2C domain.

PP2C does not seem to be evolutionary related to the main family of serine/threonine phosphatases: PP1, PP2A and PP2B . However, it is significantly similar to the catalytic subunit of pyruvate dehydrogenase phosphatase (EC 3.1.3.43) (PDPC) [4], which catalyzes dephosphorylation and concomitant reactivation of the alpha subunit of the E1 component of the pyruvate dehydrogenase complex. PDPC is a mitochondrial enzyme and, like PP2C, is magnesium-dependent.

As a signature pattern, the best conserved region was selected which is located in the N-terminal part and contains a perfectly conserved tripeptide. This region includes a conserved aspartate residue involved in divalent cation binding [5].

Consensus pattern: [LIVMFY]-[LIVMFYA]-[GSAC]-[LIVM]-[FYC]-D-G-H-[GAV]

-Note: PP2C belongs [6] to a superfamily which also includes bacterial proteins such as Bacillus spoIIE, rsbU and rsbW, Synechocystis PCC 6803 icfG as well as a domain in fungal adenylate cyclases.

09555356.091500

[1] Wenk J., Trompeter H.-I., Pettrich K.-G., Cohen P.T.W., Campbell D.G., Mieskes G. FEBS Lett. 297:135-138(1992).

[2] Maeda T., Tsai A.Y.M., Saito H. Mol. Cell. Biol. 13:5408-5417(1993).

[3] Stone J.M., Collinge M.A., Smith R.D., Horn M.A., Walker J.C. Science 266:793-795(1994).

[4] Lawson J.E., Niu X.-D., Browning K.S., Trong H.L., Yan J., Reed L.J. Biochemistry 32:8987-8993(1993).

[5] Das A.K., Helps N.R., Cohen P.T.W., Barford D. EMBO J. 24:6798-6809(1996).

[6] Bork P., Brown N.P., Hegyi H., Schultz J. Protein Sci. 5:1421-1425(1996).

419. (PPTA) Protein prenyltransferases alpha subunit repeat signature

Protein prenyltransferases catalyze the transfer of an isoprenyl moiety to a cysteine four residues from the C-terminus of several proteins. They are heterodimeric enzymes consisting of alpha and beta subunits. The alpha subunit is thought to participate in a stable complex with the isoprenyl substrate; the beta subunit binds the peptide substrate. Distinct protein prenyltransferases might share a common alpha subunit. Both the alpha and beta subunit show repetitive sequence motifs [1]. These repeats have distinct structural and functional implications and are unrelated to each other. Known protein prenyltransferase alpha subunits are:

- Mammalian protein farnesyltransferase alpha subunit.
- Yeast protein RAM2, a protein farnesyltransferase alpha subunit.
- Yeast protein BET4, a protein geranylgeranyltransferase alpha subunit.

The conserved domain of the alpha subunit consists of about 34 amino acids and is repeated five times. It contains an invariant tryptophan possibly involved in heterodimerization with the conserved phenylalanines in the repeated domains of the beta subunits, via hydrophobic bonds. The signature pattern for this domain is centered on the invariant tryptophan.

Consensus pattern: [PSIAV]-x-[NDFV]-[NEQIY]-x-[LIVMAGP]-W-[NQSTHF]-[FYHQ]-[LIVMR]

[1] Boguski M.S., Murray A.W., Powers S. New Biol. 4:408-411(1992).

09553295.061500

420. (PR55) Protein phosphatase 2A regulatory subunit PR55 signatures

Protein phosphatase 2A (PP2A) is a serine/threonine phosphatase involved in many aspects of cellular function including the regulation of metabolic enzymes and proteins involved in signal transduction. PP2A is a trimeric enzyme that consists of a core composed of a catalytic subunit associated with a 65 Kd regulatory subunit (PR65), also called subunit A; this complex then associates with a third variable subunit (subunit B), which confers distinct properties to the holoenzyme [1]. One of the forms of the variable subunit is a 55 Kd protein (PR55) which is highly conserved in mammals - where three isoforms are known to exist -, Drosophila and yeast (gene CDC55). This subunit could perform a substrate recognition function or be responsible for targeting the enzyme complex to the appropriate subcellular compartment.

As signature patterns, two perfectly conserved sequences of 15 residues were selected; one located in the N-terminal region, the other in the center of the protein.

Consensus pattern: E-F-D-Y-L-K-S-L-E-I-E-E-K-I-N

Consensus pattern: N-[AG]-H-[TA]-Y-H-I-N-S-I-S-[LIVM]-N-S-D

[1] Mayer-Jaekel R., Hemmings B.A. Trends Cell Biol. 4:287-291(1994).

421. N-(5'phosphoribosyl)anthranilate (PRA) isomerase
[1] Wilmanns M, Priestle JP, Niermann T, Jansonius JN;
J Mol Biol 1992;223:477-507.

422. (PRK) Phosphoribulokinase signature

Phosphoribulokinase (EC 2.7.1.19) (PRK) [1,2] is one of the enzymes specific to the Calvin's reductive pentose phosphate cycle which is the major route by which carbon dioxide is assimilated and reduced by autotrophic organisms. PRK catalyzes the ATP-dependent phosphorylation of ribulose 5-phosphate into ribulose 1,5-bisphosphate which is the substrate for RubisCO.
PRK's of diverse origins show different properties with respect to the size of the protein, the subunit structure, or the enzymatic regulation. However an alignment of the sequences of PRK from plants, algae, photosynthetic and chemoautotrophic bacteria shows that there are a few regions of sequence similarity. As a signature pattern one of these regions was selected.

Consensus pattern: K-[LIVM]-x-R-D-x(3)-R-G-x-[ST]-x-E

[1] Kossmann J., Klintworth R., Bowien B. Gene 85:247-252(1989).
[2] Gibson J.L., Chen J.-H., Tower P.A., Tabita F.R. Biochemistry 29:8085-8093(1990).

423. (PRPP synt) Phosphoribosyl pyrophosphate synthetase signature

Phosphoribosyl pyrophosphate synthetase (EC 2.7.6.1) (PRPP synthetase) catalyzes the formation of PRPP from ATP and ribose 5-phosphate. PRPP is then used in various biosynthetic pathways, as for example in the formation of purines, pyrimidines, histidine and tryptophan. PRPP synthetase requires inorganic phosphate and magnesium ions for its stability and activity.

In mammals, three isozymes of PRPP synthetase are found; in yeast there are at least four isozymes.

As a signature pattern for this enzyme, a very conserved region was selected that has been suggested to be involved in binding divalent cations [1]. This region contains two conserved aspartic acid residues as well as a histidine, which are all potential ligands for a cation such as magnesium.

Consensus pattern: D-[LI]-H-[SA]-x-Q-[IMST]-[QM]-G-[FY]-F-x(2)-P-[LIVMFC]-D

[1] Bower S.G., Harlow K.W., Switzer R.L., Hoven-Jensen B. J. Biol. Chem. 264:10287-10291(1989).

424. (PRTP) Herpesvirus processing and transport protein

The members of this family are associate with capsid intermediates during packaging of the virus.

Number of members: 31

[1]
Medline: 98362148
Herpes simplex virus type 1 cleavage and packaging proteins
UL15 and UL28 are associated with B but not C capsids during
packaging. Yu D, Weller SK;
J Virol 1998;72:7428-7439.

425. Photosystem I psaG / psaK (PSI PSAK) proteins signature

Photosystem I (PSI) [1] is an integral membrane protein complex that uses light energy to mediate electron transfer from plastocyanin to ferredoxin. It is found in the chloroplasts of plants and cyanobacteria. PSI is composed of at least 14 different subunits, two of which PSI-G (gene psaG) and PSI-K (gene psaK) are small hydrophobic proteins of about 7 to 9 Kd and evolutionary related [2]. Both seem to contain two transmembrane regions. Cyanobacteria seem to encode only for PSI-K.

As a signature pattern, the best-conserved region was selected which seems to correspond to the second transmembrane region.

-Consensus pattern: [GT]-F-x-[LIVM]-x-[DEA]-x(2)-[GA]-x-[GTA]-[SA]-x-G-H-x-[LIVM]-[GA]

[1] Golbeck J.H. Biochim. Biophys. Acta 895:167-204(1987).
[2] Kjaerulff S., Andersen B., Nielsen V.S., Moller B.L., Okkels J.S. J. Biol. Chem. 268:18912-18916(1993).

09535126.051402

426. PTR2 family proton/oligopeptide symporters signatures

A family of eukaryotic and prokaryotic proteins that seem to be mainly involved in the intake of small peptides with the concomitant uptake of a proton has been recently characterized [1,2]. Proteins that belong to this family are: - Fungal peptide transporter PTR2.

- Mammalian intestine proton-dependent oligopeptide transporter PeptT1.
- Mammalian kidney proton-dependent oligopeptide transporter PeptT2.
- Drosophila opt1.
- Arabidopsis thaliana peptide transporters PTR2-A and PTR2-B (also known as the histidine transporting protein NTR1).
- Arabidopsis thaliana proton-dependent nitrate/chlorate transporter CHL1.
- Lactococcus proton-dependent di- and tri-peptide transporter dtpT.
- Caenorhabditis elegans hypothetical protein C06G8.2.
- Caenorhabditis elegans hypothetical protein F56F4.5.
- Caenorhabditis elegans hypothetical protein K04E7.2.
- Escherichia coli hypothetical protein ybgH.
- Escherichia coli hypothetical protein ydgR.
- Escherichia coli hypothetical protein yhiP.
- Escherichia coli hypothetical protein yjdL.
- Bacillus subtilis hypothetical protein yclF.

These integral membrane proteins are predicted to comprise twelve transmembrane regions. As signature patterns, two of the best conserved regions were selected. The first is a region that includes the end of the second transmembrane region, a cytoplasmic loop as well as the third transmembrane region. The second pattern corresponds to the core of the fifth transmembrane region.

-Consensus pattern: [GA]-[GAS]-[LIVMFYWA]-[LIVM]-[GAS]-D-x-[LIVMFYWT]-[LIVMFYW]-G-x(3)-[TAV]-[IV]-x(3)-[GSTAV]-x-[LIVMF]-x(3)-[GA]
-Consensus pattern: [FYT]-x(2)-[LMFY]-[FYV]-[LIVMFYWA]-x-[IVG]-N-[LIVMAG]-G-[GSA]-[LIMF]

[1] Paulsen I.T., Skurray R.A. Trends Biochem. Sci. 19:404-404(1994).
[2] Steiner H.-Y., Naider F., Becker J.M. Mol. Microbiol. 16:825-834(1995).

427. Pumilio-family RNA binding domains (aka PUM-HD, Pumilio homology domain)

Puf domains are necessary and sufficient for sequence specific RNA binding in fly Pumilio and worm FBF-1 and FBF-2. Both proteins function as translational repressors in early embryonic development by binding sequences in the 3' UTR of target mRNAs (e.g. the nanos response element (NRE) in fly Hunchback mRNA, or the point mutation element (PME) in worm fem-3 mRNA). Other proteins that contain Puf domains are also plausible RNA binding proteins. JSN1_YEAST, for instance, appears to also contain a single RRM domain by HMM analysis.
Puf domains usually occur as a tandem repeat of 8 domains. The Pfam model does not necessarily recognize all 8 domains in all sequences; some sequences appear to have 5 or 6 domains on initial analysis, but further analysis suggests the presence of additional divergent domains.

[1] Zhang B, Gallegos M, Puoti A, Durkin E, Fields S, Kimble J, Wickens MP. Nature 1997;390:477-484. [2] Zamore PD, Williamson JR, Lehmann R. RNA 1997;3:1421-1433.

428. PWWP domain. The PWWP domain is named after a conserved Pro-Trp-Trp-Pro motif. The function of the domain is currently unknown. Number of members: 19

[1] Medline: 98282232. WHSC1, a 90 kb SET domain-containing gene, expressed in early development and homologous to a Drosophila dysmorphy gene maps in the Wolf-Hirschhorn syndrome critical region and is fused to IgH in t(4;14) multiple myeloma. Stec I, Wright TJ, van Ommen GJB, de Boer PAJ, van Haeringen A, Moorman AFM, Altherr MR, den Dunnen JT; Hum Mol Genet 1998;7:1071-1082.

09552925.051600

429. PX domain

Eukaryotic domain of unknown function present in phox proteins, PLD isoforms, a PI3K isoform.

Number of members: 71

[1]

Medline: 97084820

Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: binding partners of SH3 domains?

Ponting CP;

Protein Sci 1996;5:2353-2357.

430. ParA family ATPase

[1]

Medline: 91141297

A family of ATPases involved in active partitioning of diverse bacterial plasmids.

Motallebi-Veshareh M, Rouch DA, Thomas CM;

Mol Microbiol 1990;4:1455-1463.

Number of members: 122

431. (Parvo coat) Parvovirus coat protein. 72 members.

432. Pectinesterase signatures

Pectinesterase (EC 3.1.1.11) (pectin methylesterase) catalyzes the hydrolysis of pectin into pectate and methanol. In plants, it plays an important role in cell wall metabolism during fruit ripening. In plant bacterial pathogens such as Erwinia carotovora and in fungal pathogens such as Aspergillus niger, pectinesterase is involved in maceration and soft-rotting of plant tissue. Prokaryotic and eukaryotic pectinesterases share a few regions of sequence similarity [1,2,3]. two of these regions were selected as signature patterns. The first is based on a region in the N-terminal section of these enzymes; it contains a conserved tyrosine which may play a role in the catalytic mechanism [3]. The second pattern corresponds to the best conserved region, an octapeptide located in the central part of these enzymes.

-Consensus pattern: [GSTNP]-x(6)-[FYVHR]-[IVN]-[KEP]-x-G-[STIVKRQ]-Y-[DNQKRMV]-[EP]-x(3)-[LIMVA]
-Consensus pattern: [IV]-x-G-[STAD]-[LIVT]-D-[FYI]-[IV]-[FSN]-G

[1] Ray J., Knapp J., Grierson D., Bird C., Schuch W. Eur. J. Biochem. 174:119-124(1988).
[2] Plastow G.S. Mol. Microbiol. 2:247-254(1988).
[3] Markovic O., Joernvall H. Protein Sci. 1:1288-1292(1992).

433. Pentapeptide repeats (8 copies)

These repeats are found in many cyanobacterial proteins. The repeats were first identified in hglK [1]. The function of these repeats is unknown. The structure of this repeat has been predicted to be a beta-helix [2]. The repeat can be approximately described as A(D/N)LXX, where

X can be any amino acid.Number of members: 75

[1]

Medline: 96062225

The hglK gene is required for localization of heterocyst-specific glycolipids in the cyanobacterium Anabaena sp. strain PCC 7120.

Black K, Buikema WJ, Haselkorn R;

J Bacteriol 1995;177:6440-6448.

[2]Medline: 98318059

Structure and distribution of pentapeptide repeats in bacteria.

Bateman A, Murzin A, Teichmann SA;

Protein Sci 1998;7:1477-1480.

[3]Medline: 98316713

Characterisation of an Arabidopsis cDNA encoding a thylakoid lumen protein related to a novel 'pentapeptide repeat' family of proteins.

Kieselbach T, Mant A, Robinson C, Schroder WP;

FEBS Lett 1998;428:241-244.

09525656.061502

434. Polypeptide deformylase

[1]

Medline: 97002011

A new subclass of the zinc metalloproteases superfamily revealed by the solution structure of peptide deformylase.

Meinnel T, Blanquet S, Dardel F;

J Mol Biol 1996;262:375-386.

[2]Medline: 98332750

Solution structure of nickel-peptide deformylase.

Dardel F, Ragusa S, Lazennec C, Blanquet S, Meinnel T;

J Mol Biol 1998;280:501-513.

Number of members: 21

435. Peptidyl-tRNA hydrolase signatures

Peptidyl-tRNA hydrolase (EC 3.1.1.29) (PTH) is a bacterial enzyme that cleaves peptidyl-tRNA or N-acyl-aminoacyl-tRNA to yield free peptides or N-acyl-amino acids and tRNA. The natural substrate for this enzyme may be peptidyl-tRNA which drop off the ribosome during protein synthesis [1,2]. Bacterial PTH has been found [2,3] to be evolutionary related to yeast hypothetical protein YHR189w.

PTH and YHR189w are proteins of about 200 amino acid residues. As signature patterns, two conserved regions were selected that each contain an histidine. The first of these regions is located in the N-terminal section, the other in the central part.

-Consensus pattern: [FY]-x(2)-T-R-H-N-x-G-x(2)-[LIVMFA](2)-[DE]
-Consensus pattern: [GS]-x(3)-H-N-G-[LIVM]-[KR]-[DNS]-[LIVMT]

[1] Garcia-Villegas M.R., De La Vega F.M., Galindo J.M., Segura M., Buckingham R.H., Guarneros G. EMBO J. 10:3549-3555(1991).
[2] De La Vega F.M., Galindo J.M., Old I.G., Guarneros G. Gene 169:97-100(1996).
[3] Ouzounis C., Bork P., Casari G., Sander C. Protein Sci. 4:2424-2428(1995).

436. (Peptidase M17) Cytosol aminopeptidase signature

Cytosol aminopeptidase is a eukaryotic cytosolic zinc-dependent exopeptidase that catalyzes the removal of unsubstituted amino-acid residues from the N-terminus of proteins. This enzyme is often known as leucine aminopeptidase (EC 3.4.11.1) (LAP) but has been shown [1] to be identical with prolyl aminopeptidase (EC 3.4.11.5). Cytosol aminopeptidase is a hexamer of identical chains, each of which binds two zinc ions.

Cytosol aminopeptidase is highly similar to Escherichia coli pepA, a manganese dependent aminopeptidase. Residues involved in zinc ion-binding [2] in the mammalian enzyme are absolutely conserved in pepA where they presumably bind manganese.

A cytosol aminopeptidase from Rickettsia prowazekii [3] and one from Arabidopsis thaliana also belong to this family.

As a signature pattern for these enzymes, a perfectly conserved octapeptide was selected which contains two residues involved in binding metal ions: an aspartate and a glutamate.

-Consensus pattern: N-T-D-A-E-G-R-L [The D and the E are zinc/manganese ligands]

-Note: these proteins belong to family M17 in the classification of peptidases [4,E1].

[1] Matsushima M., Takahashi T., Ichinose M., Miki K., Kurokawa K., Takahashi K. Biochem. Biophys. Res. Commun. 178:1459-1464(1991).

[2] Burley S.K., David P.R., Sweet R.M., Taylor A., Lipscomb W.N. J. Mol. Biol. 224:113-140(1992).

[3] Wood D.O., Solomon M.J., Speed R.R. J. Bacteriol. 175:159-165(1993).

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

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437. Assemblin (Peptidase family S21)

[1]

Medline: 96399137

Three-dimensional structure of human cytomegalovirus protease.

Shieh HS, Kurumbail RG, Stevens AM, Stegeman RA, Sturman EJ, Pak JY, Wittwer AJ, Palmier MO, Wiegand RC, Holwerda BC, Stallings WC;

Nature 1996;383:279-282.

Number of members: 29

438. Pollen proteins Ole e I family signature

The following plant pollen proteins, whose biological function is not yet known, are structurally related [1]:

- Olive tree pollen major allergen (Ole e I).
- Tomato anther-specific protein LAT52. - Maize pollen-specific protein ZmC13.

These proteins are most probably secreted and consist of about 145 residues. As shown in the following schematic representation, there are six cysteines which are conserved in the sequence of these proteins. They seem to be involved in disulfide bonds.

xxxxxxCxCxxxxxxxxxCxxxxxxxxxxxxxxxxxCxxxxxCxxxxxxxxxxxxxxxxxxxxCxxxxxxx

******'C': conserved cysteine involved in a disulfide bond.

'*': position of the pattern.

-Consensus pattern: [EQ]-G-x-V-Y-C-D-T-C-R [The two C's are probably involved in disulfide bonds]

[1] Villalba M., Batanero E., Lopez-Otin C., Sanchez L.M., Monsalve R.I., Gonzalez De La Pena M.A., Lahoz C., Rodriguez R. Eur. J. Biochem. 216:863-869(1993).

439. Pollen allergen

This family contains allergens lol PI, PII and PIII from Lolium perenne.

Number of members: 49

[1]

Medline: 90105394

Complete primary structure of a Lolium perenne (perennial rye grass) pollen allergen, Lol p III: comparison with known Lol p I and II sequences.

Ansari AA, Shenbagamurthi P, Marsh DG;

Biochemistry 1989;28:8665-8670.

440. Porphobilinogen deaminase cofactor-binding site

Porphobilinogen deaminase (EC 4.3.1.8), or hydroxymethylbilane synthase, is an enzyme involved in the biosynthesis of porphyrins and related macrocycles. It catalyzes the assembly of four porphobilinogen (PBG) units in a head to tail fashion to form hydroxymethylbilane.

The enzyme covalently binds a dipyrromethane cofactor to which the PBG subunits are added in a stepwise fashion. In the Escherichia coli enzyme (gene hemC), this cofactor has been shown [1] to be bound by the sulfur atom of a cysteine. The region around this cysteine is conserved in porphobilinogen deaminases from various prokaryotic and eukaryotic sources.

-Consensus pattern: E-R-x-[LIVMFA]-x(3)-[LIVMF]-x-G-[GSA]-C-x-[IVT]-P-[LIVMF]-[GSA] [C is the cofactor attachment site]

[1] Miller A.D., Hart G.J., Packman L.C., Battersby A.R. Biochem. J. 254:915-918(1988).

09553531.061500

441. Presenilin

Mutations in presenilin-1 are a major cause of early onset Alzheimer's disease [2]. It has been found that presenilin-1 (Swiss:P49768) binds to beta-catenin in vivo [4]. This family also contains SPE proteins from C.elegans.

Number of members: 23

[1]

Medline: 98045995

Presenilins and Alzheimer's disease.

Kim TW, Tanzi RE;

Curr Opin Neurobiol 1997;7:683-688.

[2]Medline: 98045995

Presenilins and Alzheimer's disease.

Kim TW, Tanzi RE;

Curr Opin Neurobiol 1997;7:683-688.

[3]Medline: 98099802

Interaction of presenilins with the filamin family of actin-binding proteins.

Zhang W, Han SW, McKeel DW, Goate A, Wu JY;

J Neurosci 1998;18:914-922.

[4]Medline: 99004850

Destabilisation of beta-catenin by mutations in presenilin-1 potentiates neuronal apoptosis.

Zhang Z, Hartmann H, Do VM, Abramowski D, Sturchler-Pierrat C, Staufenbiel M, Sommer B, van de Wetering M, Clevers H, Saftig P, De Strooper B, He X, Yankner BA;

Nature 1998;395:698-702.

442. (Pribosyltran) Purine/pyrimidine phosphoribosyl transferases signature

Phosphoribosyltransferases (PRT) are enzymes that catalyze the synthesis of beta-n-5'-monophosphates from phosphoribosylpyrophosphate (PRPP) and an enzyme specific amine. A number of PRT's are involved in the biosynthesis of purine, pyrimidine, and pyridine nucleotides, or in the salvage of purines and pyrimidines. These enzymes are:

- Adenine phosphoribosyltransferase (EC 2.4.2.7) (APRT), which is involved in purine salvage.
- Hypoxanthine-guanine or hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) (HGPRT or HPRT), which are involved in purine salvage.
- Orotate phosphoribosyltransferase (EC 2.4.2.10) (OPRT), which is involved in pyrimidine biosynthesis.
- Amido phosphoribosyltransferase (EC 2.4.2.14), which is involved in purine biosynthesis.
- Xanthine-guanine phosphoribosyltransferase (EC 2.4.2.22) (XGPRT), which is involved in purine salvage.

In the sequence of all these enzymes there is a small conserved region which may be involved in the enzymatic activity and/or be part of the PRPP binding site [1].

-Consensus pattern: [LIVMFYWCTA]-[LIVM]-[LIVMA]-[LIVMFC]-[DE]-D-[LIVMS]-[LIVM]-[STAVD]-[STAR]-[GAC]-x-[STAR]

-Note: in position 11 of the pattern most of these enzymes have Gly.

[1] Hershey H.V., Taylor M.W. Gene 43:287-293(1986).

09559526.051500

443. (Pro CA)

Prokaryotic-type carbonic anhydrases signatures

Carbonic anhydrases (EC 4.2.1.1) (CA) are zinc metalloenzymes which catalyze the reversible hydration of carbon dioxide. In Escherichia coli, CA (gene cynT) is involved in recycling carbon dioxide formed in the bicarbonate-dependent decomposition of cyanate by cyanase (gene cynS). By this action, it prevents the depletion of cellular bicarbonate [1]. In photosynthetic bacteria and plant chloroplast, CA is essential to inorganic carbon fixation [2]. Prokaryotic and plant chloroplast CA are structurally and evolutionary related and form a family distinct from the one which groups the many different forms of eukaryotic CA's (see <PDOC00146>). Hypothetical proteins yadF from Escherichia coli and HI1301 from Haemophilus influenzae also belong to this family. Two signature patterns were developed for this family of enzymes. Both patterns contain conserved residues that could be involved in binding zinc (cysteine and histidine).

-Consensus pattern: C-[SA]-D-S-R-[LIVM]-x-[AP]

-Consensus pattern: [EQ]-Y-A-[LIVM]-x(2)-[LIVM]-x(4)-[LIVMF](3)-x-G-H-x(2)-C-G

[1] Guilloton M.B., Korte J.J., Lamblin A.F., Fuchs J.A., Anderson P.M. J. Biol. Chem. 267:3731-3734(1992).

[2] Fukuzawa H., Suzuki E., Komukai Y., Miyachi S. Proc. Natl. Acad. Sci. U.S.A. 89:4437-4441(1992).

444. (Prolyl_oligopep)

Prolyl oligopeptidase family serine active site

The prolyl oligopeptidase family [1,2,3] consist of a number of evolutionary related peptidases whose catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. The known members of this family are listed below.

- Prolyl endopeptidase (EC 3.4.21.26) (PE) (also called post-proline cleaving enzyme). PE is an enzyme that cleaves peptide bonds on the C-terminal side of prolyl residues. The sequence of PE has been obtained from a mammalian species (pig) and from bacteria (Flavobacterium meningosepticum and Aeromonas hydrophila); there is a high degree of sequence conservation between these sequences.
- Escherichia coli protease II (EC 3.4.21.83) (oligopeptidase B) (gene prtB) which cleaves peptide bonds on the C-terminal side of lysyl and argininyl residues.
- Dipeptidyl peptidase IV (EC 3.4.14.5) (DPP IV). DPP IV is an enzyme that removes N-terminal dipeptides sequentially from polypeptides having unsubstituted N-termini provided that the penultimate residue is proline.
- Yeast vacuolar dipeptidyl aminopeptidase A (DPAP A) (gene: STE13) which is responsible for the proteolytic maturation of the alpha-factor precursor.
- Yeast vacuolar dipeptidyl aminopeptidase B (DPAP B) (gene: DAP2).
- Acylamino-acid-releasing enzyme (EC 3.4.19.1) (acyl-peptide hydrolase).

This enzyme catalyzes the hydrolysis of the amino-terminal peptide bond of an N-acetylated protein to generate a N-acetylated amino acid and a protein with a free amino-terminus.

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A conserved serine residue has experimentally been shown (in E.coli proteaseII as well as in pig and bacterial PE) to be necessary for the catalytic mechanism. This serine, which is part of the catalytic triad (Ser, His, Asp), is generally located about 150 residues away from the C-terminal extremity of these enzymes (which are all proteins that contains about 700 to 800 amino acids).

Consensus pattern: D-x(3)-A-x(3)-[LIVMFYW]-x(14)-G-x-S-x-G-G-[LIVMFYW](2) [S is the active site residue] Sequences known to belong to this class detected by the pattern ALL, except for yeast DPAP A.

Note: these proteins belong to families S9A/S9B/S9C in the classification of peptidases [4].

[1] Rawlings N.D., Polgar L., Barrett A.J. Biochem. J. 279:907-911(1991).

[2] Barrett A.J., Rawlings N.D.

□

[3] Polgar L., Szabo E.

□

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

445. (Pterin 4a)

Pterin 4 alpha carbinolamine dehydratase

Pterin 4 alpha carbinolamine dehydratase is aka DCoH (dimerisation cofactor of hepatocyte nuclear factor 1-alpha).

Number of members: 11

[1] Cronk JD, Endrizzi JA, Alber T; Medline: 97052967 “High-resolution structures of the bifunctional enzyme and transcriptional coactivator DCoH and its complex with a product analogue.” Protein Sci 1996;5:1963-1972.

446. (Pyridox oxidase)

Pyridoxamine 5'-phosphate oxidase signature

Pyridoxamine 5'-phosphate oxidase (EC 1.4.3.5) is a FMN flavoprotein involved in the de novo synthesis of pyridoxine (vitamin B6) and pyridoxal phosphate. It oxidizes pyridoxamine-5-P (PMP) and pyridoxine-5-P (PNP) to pyridoxal-5-P. The sequences of the enzyme from bacterial (genes pdxH or fprA) [1] and fungal (gene PDX3) [2] sources show that this protein has been highly conserved throughout evolution.
PdxH is evolutionary related [3] to one of the enzymes in the phenazine biosynthesis protein pathway, phzD (also known as phzG). As a signature pattern, a highly conserved region was selected located in the C-terminal part of these enzymes.

-Consensus pattern: [LIVF]-E-F-W-[QHG]-x(4)-R-[LIVM]-H-[DNE]-R

[1] Lam H.-M., Winkler M.E. J. Bacteriol. 174:6033-6045(1992).
[2] Loubbardi A., Karst F., Guilloton M., Marcireau C. J. Bacteriol. 177:1817-1823(1995).
[3] Pierson L.S. III, Gaffney T., Lam S., Gong F. FEMS Microbiol. Lett. 134:299-307(1995).

447. (Pyrophosphatase)
Inorganic pyrophosphatase signature

Inorganic pyrophosphatase (EC 3.6.1.1) (PPase) [1,2] is the enzyme responsible for the hydrolysis of pyrophosphate (PPi) which is formed principally as the product of the many biosynthetic reactions that utilize ATP. All known Ppases require the presence of divalent metal cations, with magnesium conferring the highest activity. Among other residues, a lysine has been postulated to be part or close to the active site. PPases have been sequenced from bacteria such as Escherichia coli (homohexamer), thermophilic bacteria PS-3 and Thermus thermophilus, from the archaebacteria Thermoplasma acidophilum, from fungi (homodimer), from a plant, and from bovine retina. In yeast, a mitochondrial isoform of PPase has been characterized which seems to be involved in energy production and whose activity is stimulated by uncouplers of ATP synthesis.

The sequences of PPases share some regions of similarities. As signature patterns a region was selected that contains three conserved aspartates that are involved in the binding of cations.

-Consensus pattern: D-[SGDN]-D-[PE]-[LIVMF]-D-[LIVMGAC]
[The three D's bind divalent metal cations]

[1] Lahti R., Kolakowski L.F. Jr., Heinonen J., Vihinen M., Pohjanoksa K., Cooperman B.S. Biochim. Biophys. Acta 1038:338-345(1990).
[2] Cooperman B.S., Baykov A.A., Lahti R. Trends Biochem. Sci. 17:262-266(1992).

448. (Peptidase S26)

Signal peptidases I signatures.

Signal peptidases (SPases) [1] (aka leader peptidases) remove the signal peptides from secretory proteins. In prokaryotes three types of SPasesare known: type I (gene lepB) which is responsible for the processing of the majority of exported pre-proteins; type II (gene lsp) which only process lipoproteins, and a third type involved in the processing of pili subunits. SPase I (EC 3.4.21.89) is an integral membrane protein that is anchored in the cytoplasmic membrane by one (in B. subtilis) or two (in E. coli) N-terminal transmembrane domains with the main part of the protein protuding in the periplasmic space. Two residues have been shown [2,3] to be essential for the catalytic activity of SPase I: a serine and an lysine. SPase I is evolutionary related to the yeast mitochondrial inner membrane protease subunit 1 and 2 (genes IMP1 and IMP2) which catalyze the removal of signal peptides required for the targeting of proteins from the mitochondrial matrix, across the inner membrane, into the inter-membrane space [4].In eukaryotes the removal of signal peptides is effected by an oligomeric enzymatic complex composed of at least five subunits: the signal peptidase complex (SPC). The SPC is located in the endoplasmic reticulum membrane. Two components of mammalian SPC, the 18 Kd (SPC18) and the 21 Kd (SPC21) subunits as well as the yeast SEC11 subunit have been shown [5] to share regions of sequence similarity with prokaryotic SPases I and yeast IMP1/IMP2. Three signature patterns have been developed for these proteins. The first signature contains the putative active site serine, the second signature contains the putative active site lysine which is not conserved in the SPC subunits, and the third signature corresponds to a conserved region of unknown biological significance which is located in the C-terminal section of all these proteins.

Consensus pattern: [GS]-x-S-M-x-[PS]-[AT]-[LF] [S is an active site residue]-
Consensus pattern: K-R-[LIVMSTA](2)-G-x-[PG]-G-[DE]-x-[LIVM]-x-[LIVMFY] [K is an active site residue]-
Consensus pattern: [LIVMFYW](2)-x(2)-G-D-[NH]-x(3)-[SND]-x(2)-[SG]-

[1] Dalbey R.E., von Heijne G. Trends Biochem. Sci. 17:474-478(1992).[2] Sung M., Dalbey R.E. J. Biol. Chem. 267:13154-13159(1992).[3] Black M.T. J. Bacteriol. 175:4957-4961(1993).[4] Nunnari J., Fox T.D., Walter P. Science 262:1997-2004(1993).[5] van Dijl

J.M., de Jong A., Vehmaanpera J., Venema G., Bron S. EMBO J. 11:2819-2828(1992).[6] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]

449. (Peptidase C1) Eukaryotic thiol (cysteine) proteases active sites. Eukaryotic thiol proteases (EC 3.4.22.-) [1] are a family of proteolytic enzymes which contain an active site cysteine. Catalysis proceeds through a thioester intermediate and is facilitated by a nearby histidine side chain; an asparagine completes the essential catalytic triad. The proteases which are currently known to belong to this family are listed below (references are only provided for recently determined sequences). - Vertebrate lysosomal cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), L (EC 3.4.22.15), and S (EC 3.4.22.27) [2]. - Vertebrate lysosomal dipeptidyl peptidase I (EC 3.4.14.1) (also known as cathepsin C) [2]. - Vertebrate calpains (EC 3.4.22.17). Calpains are intracellular calcium- activated thiol protease that contain both a N-terminal catalytic domain and a C-terminal calcium-binding domain. - Mammalian cathepsin K, which seems involved in osteoclastic bone resorption [3]. - Human cathepsin O [4]. - Bleomycin hydrolase. An enzyme that catalyzes the inactivation of the antitumor drug BLM (a glycopeptide). - Plant enzymes: barley aleurain (EC 3.4.22.16), EP-B1/B4; kidney bean EP-C1, rice bean SH-EP; kiwi fruit actinidin (EC 3.4.22.14); papaya latex papain (EC 3.4.22.2), chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30), and proteinase IV (EC 3.4.22.25); pea turgor-responsive protein 15A; pineapple stem bromelain (EC 3.4.22.32); rape COT44; rice oryzain alpha, beta, and gamma; tomato low-temperature induced, Arabidopsis thaliana A494, RD19A and RD21A. - House-dust mites allergens DerP1 and EurM1. - Cathepsin B-like proteinases from the worms Caenorhabditis elegans (genes gcp-1, cpr-3, cpr-4, cpr-5 and cpr-6), Schistosoma mansoni (antigen SM31) and Japonica (antigen SJ31), Haemonchus contortus (genes AC-1 and AC-2), and Ostertagia ostertagi (CP-1 and CP-3). - Slime mold cysteine proteinases CP1 and CP2. - Cruzipain from Trypanosoma cruzi and brucei. - Throphozoite cysteine proteinase (TCP) from various Plasmodium species. - Proteases from Leishmania mexicana, Theileria annulata and Theileria parva. - Baculoviruses cathepsin-like enzyme (v-cath). - Drosophila small optic lobes protein (gene sol), a neuronal protein that contains a calpain-like domain. - Yeast thiol protease BLH1/YCP1/LAP3. - Caenorhabditis elegans hypothetical protein C06G4.2, a calpain-like protein. Two bacterial peptidases are also part of this family: - Aminopeptidase C from Lactococcus lactis (gene pepC) [5]. - Thiol protease tpr from Porphyromonas gingivalis.

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Three other proteins are structurally related to this family, but may have lost their proteolytic activity. - Soybean oil body protein P34. This protein has its active site cysteine replaced by a glycine. - Rat testin, a sertoli cell secretory protein highly similar to cathepsin L but with the active site cysteine is replaced by a serine. Rat testin should not be confused with mouse testin which is a LIM-domain protein (see <PDOC00382>). - Plasmodium falciparum serine-repeat protein (SERA), the major blood stage antigen. This protein of 111 Kd possesses a C-terminal thiol-protease-like domain [6], but the active site cysteine is replaced by a serine. The sequences around the three active site residues are well conserved and can be used as signature patterns.

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Consensus pattern: Q-x(3)-[GE]-x-C-[YW]-x(2)-[STAGC]-[STAGCV] [C is the active site residue]- Note: the residue in position 4 of the pattern is almost always cysteine; the only exceptions are calpains (Leu), bleomycin hydrolase (Ser) and yeast YCP1 (Ser). -Note: the residue in position 5 of the pattern is always Gly except in papaya protease IV where it is Glu.

Consensus pattern: [LIVMGSTAN]-x-H-[GSACE]-[LIVM]-x-[LIVMAT](2)-G-x-[GSADNH] [H is the active site residue]-

Consensus pattern: [FYCH]-[WI]-[LIVT]-x-[KRQAG]-N-[ST]-W-x(3)-[FYW]-G-x(2)-G-[LFYW]-[LIVMFYG]-x-[LIVMF] [N is the active site residue] - Note: these proteins belong to family C1 (papain-type) and C2 (calpains) in the classification of peptidases [7,E1].-

[1] Dufour E. Biochimie 70:1335-1342(1988).[2] Kirschke H., Barrett A.J., Rawlings N.D. Protein Prof. 2:1587-1643(1995).[3] Shi G.-P., Chapman H.A., Bhairi S.M., Deleeuw C., Reddy V.Y., Weiss S.J. FEBS Lett. 357:129-134(1995).[4] Velasco G., Ferrando A.A., Puente X.S., Sanchez L.M., Lopez-Otin C. J. Biol. Chem. 269:27136-27142(1994).[5] Chapot-Chartier M.P., Nardi M., Chopin M.C., Chopin A., Gripon J.C. Appl. Environ. Microbiol. 59:330-333(1993).[6] Higgins D.G., McConnell D.J., Sharp P.M. Nature 340:604-604(1989).[7] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

450. (peptidase M24) Aminopeptidase P and proline dipeptidase signature (1). Aminopeptidase P (EC 3.4.11.9) is the enzyme responsible for the release of any N-terminal amino acid adjacent to a proline residue. Proline dipeptidase(EC 3.4.13.9) (prolidase) splits

dipeptides with a prolyl residue in the carboxyl terminal position. Bacterial aminopeptidase P II (gene pepP) [1], proline dipeptidase (gene pepQ)[2], and human proline dipeptidase (gene PEPD) [3] are evolutionary related. These proteins are manganese metalloenzymes. Yeast hypothetical proteins YER078c and YFR006w and Mycobacterium tuberculosis hypothetical protein MtCY49.29c also belong to this family. As a signature pattern for these enzymes a conserved region that contains three histidine residues has been developed

Consensus pattern: [HA]-[GSYR]-[LIVMT]-[SG]-H-x-[LIV]-G-[LIVM]-x-[IV]-H-[DE]-

[1] Yoshimoto T., Tone H., Honda T., Osatomi K., Kobayashi R., Tsuru D. J. Biochem. 105:412-416(1989).[2] Nakahigashi K., Inokuchi H. Nucleic Acids Res. 18:6439-6439(1990).[3] Endo F., Tanoue A., Nakai H., Hata A., Indo Y., Titani K., Matsuda I. J. Biol. Chem. 264:4476-4481(1989).[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

Methionine aminopeptidase signatures. (2). Methionine aminopeptidase (EC 3.4.11.18) (MAP) is responsible for the removal of the amino-terminal (initiator) methionine from nascent eukaryotic cytosolic and cytoplasmic prokaryotic proteins if the penultimate amino acid is small and uncharged. All MAP studied to date are monomeric proteins that require cobalt ions for activity. Two subfamilies of MAP enzymes are known to exist [1,2]. While being evolutionary related, they only share a limited amount of sequence similarity mostly clustered around the residues shown, in the Escherichia coli MAP [3],to be involved in cobalt-binding. The first family consists of enzymes from prokaryotes as well as eukaryoticMAP-1, while the second group is made up of archebacterial MAP and eukaryoticMAP-2. The second subfamily also includes proteins which do not seem to be MAP, but that are clearly evolutionary related such as mouse proliferation-associated protein 1 and fission yeast curved DNA-binding protein. For each of these subfamilies, a specific signature pattern that includes residues known to be involved in colbalt-binding has been developed.

Consensus pattern: [MFY]-x-G-H-G-[LIVMC]-[GSH]-x(3)-H-x(4)-[LIVM]-x-[HN]- [YWV] [H is a cobalt ligand]-

Consensus pattern: [DA]-[LIVMY]-x-K-[LIVM]-D-x-G-x-[HQ]-[LIVM]-[DNS]-G-x(3)-[DN] [The second D and the last D/N are cobalt ligands]

[1] Arfin S.M., Kendall R.L., Hall L., Weaver L.H., Stewart A.E., Matthews B.W., Bradshaw R.A. Proc. Natl. Acad. Sci. U.S.A. 92:7714-7718(1995).[2] Keeling P.J., Doolittle W.F. Trends Biochem. Sci. 21:285-286(1996).[3] Roderick S.L., Mathews B.W. Biochemistry 32:3907-3912(1993).[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

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451. Cytochrome P450 cysteine heme-iron ligand signature

Cytochrome P450's [1,2,3,<u>E1</u>] are a group of enzymes involved in the oxidative metabolism of a high number of natural compounds (such as steroids, fatty acids, prostaglandins, leukotrienes, etc) as well as drugs, carcinogens and mutagens. Based on sequence similarities, P450's have been classified into about forty different families [4,5]. P450's are proteins of 400 to 530 amino acids; the only exception is Bacillus BM-3 (CYP102) which is a protein of 1048residues that contains a N-terminal P450 domain followed by a reductase domain. P450's are heme proteins. A conserved cysteine residue in the C-terminal part of P450's is involved in binding the heme iron in the fifth coordination site. From a region around this residue, a ten residue signature was developed specific to P450's.

Consensus pattern: [FW]-[SGNH]-x-[GD]-x-[RHPT]-x-C-[LIVMFAP]-[GAD] [C is the heme iron ligand]-

[1] Nebert D.W., Gonzalez F.J. Annu. Rev. Biochem. 56:945-993(1987).
[2] Coon M.J., Ding X., Pernecky S.J., Vaz A.D.N. FASEB J. 6:669-673(1992).
[3] Guengerich F.P. J. Biol. Chem. 266:10019-10022(1991).
[4] Nelson D.R., Kamataki T., Waxman D.J., Guengerich F.P., Estrabrook R.W., Feyereisen R., Gonzalez F.J., Coon M.J., Gunsalus I.C., Gotoh O., Okuda K., Nebert D.W. DNA Cell Biol. 12:1-51(1993).
[5] Degtyarenko K.N., Archakov A.I. FEBS Lett. 332:1-8(1993).

452. (Pec Lyase) Pectate lyase

This enzyme forms a right handed beta helix structure. Pectate lyase is an enzyme involved in the maceration and soft rotting of plant tissue.

[1] Yoder MD, Keen NT, Jurnak F, Science 1993;260:1503-1507.

453. (pep M24) Aminopeptidase P and proline dipeptidase signature (pep1)

Aminopeptidase P (EC 3.4.11.9) is the enzyme responsible for the release of any N-terminal amino acid adjacent to a proline residue. Proline dipeptidase(EC 3.4.13.9) (prolidase) splits dipeptides with a prolyl residue in the carboxyl terminal position. Bacterial aminopeptidase P II (gene pepP) [1], proline dipeptidase (gene pepQ)[2], and human proline dipeptidase (gene PEPD) [3] are evolutionary related. These proteins are manganese metalloenzymes. Yeast hypothetical proteins YER078c and YFR006w and Mycobacterium tuberculosis .hypothetical protein MtCY49.29c also belong to this family. As a signature pattern for these enzymes a conserved region was selected that contains three histidine residues.

Consensus pattern: [HA]-[GSYR]-[LIVMT]-[SG]-H-x-[LIV]-G-[LIVM]-x-[IV]-H-[DE]-

[1] Yoshimoto T., Tone H., Honda T., Osatomi K., Kobayashi R., Tsuru D. J. Biochem. 105:412-416(1989).

[2] Nakahigashi K., Inokuchi H. Nucleic Acids Res. 18:6439-6439(1990).

[3] Endo F., Tanoue A., Nakai H., Hata A., Indo Y., Titani K., Matsuda I. J. Biol. Chem. 264:4476-4481(1989).

[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

Methionine aminopeptidase signatures (pep2)

Methionine aminopeptidase (EC 3.4.11.18) (MAP) is responsible for the removal of the amino-terminal (initiator) methionine from nascent eukaryotic cytosolic and cytoplasmic prokaryotic proteins if the penultimate amino acid is small and uncharged. All MAP studied to date are monomeric proteins that require cobalt ions for activity. Two subfamilies of MAP enzymes are known to exist [1,2]. While being evolutionary related, they only share a limited amount of sequence similarity mostly clustered around the residues shown, in the Escherichia coli MAP [3],to be involved in cobalt-binding. The first family consists of enzymes from prokaryotes as well as eukaryotic MAP-1, while the second group is made up of archebacterial MAP and eukaryotic MAP-2. The second subfamily also includes proteins which do not seem to be MAP, but that are clearly evolutionary related such as mouse proliferation-associated protein 1 and fission yeast curved DNA-binding protein. For each of these subfamilies, a specific signature pattern was developed that includes residues known to be involved in colbalt-binding.

Consensus pattern: [MFY]-x-G-H-G-[LIVMC]-[GSH]-x(3)-H-x(4)-[LIVM]-x-[HN]- [YWV] [H is a cobalt ligand]-

Consensus pattern: [DA]-[LIVMY]-x-K-[LIVM]-D-x-G-x-[HQ]-[LIVM]-[DNS]-G-x(3)-[DN] [The second D and the last D/N are cobalt ligands]

[1] Arfin S.M., Kendall R.L., Hall L., Weaver L.H., Stewart A.E., Matthews B.W., Bradshaw R.A. Proc. Natl. Acad. Sci. U.S.A. 92:7714-7718(1995).
[2] Keeling P.J., Doolittle W.F. Trends Biochem. Sci. 21:285-286(1996).
[3] Roderick S.L., Mathews B.W. Biochemistry 32:3907-3912(1993).
[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

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454. Peroxidases signatures

Peroxidases (EC 1.11.1.-) [1] are heme-binding enzymes that carry out a variety of biosynthetic and degradative functions using hydrogen peroxide as the electron acceptor. Peroxidases are widely distributed throughout bacteria, fungi, plants, and vertebrates. In peroxidases the heme prosthetic group is protoporphyrin IX and the fifth ligand of the heme iron is a histidine (known as the proximal histidine). Another histidine residue (the distal histidine) serves as an acid-base catalyst in the reaction between hydrogen peroxide and the enzyme. The regions around these two active site residues are more or less conserved in a majority of peroxidases [2,3]. The enzymes in which one or both of these regions can be found are listed below. - Yeast cytochrome c peroxidase (EC 1.11.1.5). - Myeloperoxidase (EC 1.11.1.7) (MPO). MPO is found in granulocytes and monocytes and plays a major role in the oxygen-dependent microbicidal system of neutrophils. - Lactoperoxidase (EC 1.11.1.7) (LPO). LPO is a milk protein which acts as an antimicrobial agent. - Eosinophil peroxidase (EC 1.11.1.7) (EPO). An enzyme found in the cytoplasmic granules of eosinophils. - Thyroid peroxidase (EC 1.11.1.8) (TPO). TPO plays a central role in the biosynthesis of thyroid hormones. It catalyzes the iodination and coupling of the hormonogenic tyrosines in thyroglobulin to yield the thyroid hormones T3 and T4. - Fungal ligninases. Ligninase catalyzes the first step in the degradation of lignin. It depolymerizes lignin by catalyzing the C(alpha)-C(beta) cleavage of the propyl side chains of lignin. - Plant peroxidases (EC 1.11.1.7). Plants expresses a large numbers of isozymes of peroxidases. Some of them play a role in cell-suberization by catalyzing the deposition of the aromatic residues of suberin on the cell wall, some are expressed as a defense response toward wounding, others are involved in the metabolism of auxin and the biosynthesis of lignin. - Prokaryotic catalase-peroxidases. Some bacterial species produce enzymes that exhibit both catalase and broad-spectrum peroxidase activities [4]. Examples of such enzymes are: catalase HP I from Escherichia coli (gene katG) and perA from Bacillus stearothermophilus.

Consensus pattern: [DET]-[LIVMTA]-x(2)-[LIVM]-[LIVMSTAG]-[SAG]-[LIVMSTAG]-H-[STA]-[LIVMFY] [H is the proximal heme-binding ligand] -
Consensus pattern: [SGATV]-x(3)-[LIVMA]-R-[LIVMA]-x-[FW]-H-x-[SAC] [H is an active site residue]-

[1] Dawson J.H. Science 240:433-439(1988).
[2] Kimura S., Ikeda-Saito M. Proteins 3:113-120(1988).
[3] Henrissat B., Saloheimo M., Lavaitte S., Knowles J.K.C. Proteins 8:251-257(1990).
[4] Welinder K.G. Biochim. Biophys. Acta 1080:215-220(1991).

455. pfkB family of carbohydrate kinases signatures
It has been shown [1,2,3] that the following carbohydrate and purine kinasesare evolutionary related and can be grouped into a single family, which isknown [1] as the 'pfkB family': - Fructokinase (EC 2.7.1.4) (gene scrK). - 6-phosphofructokinase isozyme 2 (EC 2.7.1.11) (phosphofructokinase-2) (gene pfkB). pfkB is a minor phosphofructokinase isozyme in Escherichia coli and is not evolutionary related to the major isozyme (gene pfkA). Plants 6-phosphofructokinase also belong to this family. - Ribokinase (EC 2.7.1.15) (gene rbsK). - Adenosine kinase (EC 2.7.1.20) (gene ADK). - 2-dehydro-3-deoxygluconokinase (EC 2.7.1.45) (gene: kdgK). - 1-phosphofructokinase (EC 2.7.1.56) (fructose 1-phosphate kinase)

(gene fruK). - Inosine-guanosine kinase (EC 2.7.1.73) (gene gsk). - Tagatose-6-phosphate kinase (EC 2.7.1.144) (phosphotagatokinase) (gene lacC). - Escherichia coli hypothetical protein yeiC. - Escherichia coli hypothetical protein yeiI. - Escherichia coli hypothetical protein yhfQ. - Escherichia coli hypothetical protein yihV. - Bacillus subtilis hypothetical protein yxdC. - Yeast hypothetical protein YJR105w.All the above kinases are proteins of from 280 to 430 amino acid residues that share a few region of sequence similarity. Two of these regions were selected as signature patterns. The first pattern is based on a region rich in glycine which is located in the N-terminal section of these enzymes; while the second pattern is based on a conserved region in the C-terminal section.

Consensus pattern: [AG]-G-x(0,1)-[GAP]-x-N-x-[STA]-x(6)-[GS]-x(9)-G-

Consensus pattern: [DNSK]-[PSTV]-x-[SAG](2)-[GD]-D-x(3)-[SAGV]-[AG]- [LIVMFYA]-[LIVMSTAP]

[1] Wu L.-F., Reizer A., Reizer J., Cai B., Tomich J.M., Saier M.H. Jr. J. Bacteriol. 173:3117-3127(1991).
[2] Orchard L.M.D., Kornberg H.L. Proc. R. Soc. Lond., B, Biol. Sci. 242:87-90(1990).
[3] Blatch G.L., Scholle R.R., Woods D.R. Gene 95:17-23(1990).

456. Phospholipase A2 active sites signatures

Phospholipase A2 (EC 3.1.1.4) (PA2) [1,2] is an enzyme which releases fatty acids from the second carbon group of glycerol. PA2's are small and rigid proteins of 120 amino-acid residues that have four to seven disulfide bonds.PA2 binds a calcium ion which is required for activity. The side chains of two conserved residues, a histidine and an aspartic acid, participate in a 'catalytic network'. Many PA2's have been sequenced from snakes, lizards, bees and mammals. In the latter, there are at least four forms: pancreatic, membrane-associated as well as two less characterized forms. The venom of most snakes contains multiple forms of PA2. Some of them are presynaptic neurotoxins which inhibit neuromuscular transmission by blocking acetylcholine release from the nerve termini. Two different signature patterns were derived for PA2's. The first is centered on the active site histidine and contains three cysteines involved in disulfide bonds. The second is centered on the active site aspartic acid and also contains three cysteines involved in disulfide bonds.

Consensus pattern: C-C-x(2)-H-x(2)-C [H is the active site residue] This pattern will not detect some snake toxins homologous with PA2 but which have lost their catalytic activity as well as otoconin-22, a Xenopus protein from the aragonitic otoconia which is also unlikely to be enzymatically active.

Consensus pattern: [LIVMA]-C-{LIVMFYWPCST}-C-D-x(5)-C [D is the active site residue] The majority of functional and non-functional PA2's. Undetected sequences are bee PA2, gila monster PA2's, PA2 PL-X from habu and PA2 PA-5 from mulga.

[1] Davidson F.F., Dennis E.A. J. Mol. Evol. 31:228-238(1990).

[2] Gomez F., Vandermeers A., Vandermeers-Piret M.-C., Herzog R., Rathe J., Stievenart M., Winand J., Christophe J. Eur. J. Biochem. 186:23-33(1989).

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457. Phosphorylase pyridoxal-phosphate attachment site. Phosphorylases (EC 2.4.1.1) [1] are important allosteric enzymes in carbohydrate metabolism. They catalyze the formation of glucose 1-phosphatefrom polyglucose such as glycogen, starch or maltodextrin. Enzymes from different sources differ in their regulatory mechanisms and their natural substrates. However, all known phosphorylases share catalytic and structural properties. They are pyridoxal-phosphate dependent enzymes; the pyridoxal-P group is attached to a lysine residue around which the sequence is highly conserved and can be used as a signature pattern to detect this class of enzymes.

Consensus pattern: E-A-[SC]-G-x-[GS]-x-M-K-x(2)-[LM]-N [K is the pyridoxal-P attachment site]-

[1] Fukui T., Shimomura S., Nakano K. Mol. Cell. Biochem. 42:129-144(1982).

458. Protein kinases signatures and profile

Eukaryotic protein kinases [1 to 5] are enzymes that belong to a very extensive family of proteins which share a conserved catalytic core common toboth serine/threonine and tyrosine protein kinases. There are a number ofconserved regions in the catalytic domain of protein kinases. Two of these regions were selected to build signature patterns. The first region, which is located in the N-terminal extremity of the catalytic domain, is a glycine-rich stretch of residues in the vicinity of a lysine residue, which has been shown to be involved in ATP binding. The second region, which is located in the central part of the catalytic domain, contains a conserved aspartic acid residue which is important for the catalytic activity of the enzyme [6]; Two signature patterns were derived for that region: one specific for serine/threonine kinases and the other for tyrosine kinases. A profile was also developed which is based on the alignment in [1] and covers the entire catalytic domain.

Consensus pattern: [LIV]-G-{P}-G-{P}-[FYWMGSTNH]-[SGA]-{PW}-[LIVCAT]-{PD}-x-[GSTACLIVMFY]-x(5,18)-[LIVMFYWCSTAR]-[AIVP]-[LIVMFAGCKR]-K [K binds ATP]. The majority of known protein kinases belong to the class detected by this pattern, but it fails to find a number of them, especially viral kinases which are quite divergent in this region and are completely missed by this pattern.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3) [D is an active site residue]. Most serine/ threonine specific protein kinases belong to this class detected by the pattern with 10 exceptions (half of them viral kinases) and also Epstein-Barr virus BGLF4 and Drosophila ninaC which have respectively Ser and Arg instead of the conserved Lys and which are therefore detected by the tyrosine kinase specific pattern described below.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-[RSTAC]-x(2)-N-[LIVMFYC](3) [D is an active site residue] ALL tyrosine specific protein kinases with the exception of human ERBB3 and mouse blk belong to this class detected by the pattern. This pattern will also detect most bacterial aminoglycoside phosphotransferases [8,9] and herpesviruses gangciclovir kinases [10]; which are proteins structurally and evolutionary related to protein kinases. This profile also detects receptor guanylate cyclases and 2-5A-dependent ribonucleases. Sequence similarities between these two families and the eukaryotic protein kinase family have been noticed before. It also detects Arabidopsis thaliana kinase- like protein TMKL1 which seems to have lost its catalytic activity. If a protein analyzed includes the two protein kinase signatures, the probability of it being a protein kinase is close to 100%. Eukaryotic-type protein kinases have also been found in prokaryotes such as Myxococcus xanthus [11] and Yersinia pseudotuberculosis.

09595326 .061502

[1] Hanks S.K., Hunter T. FASEB J. 9:576-596(1995).

[2] Hunter T. Meth. Enzymol. 200:3-37(1991).

[3] Hanks S.K., Quinn A.M. Meth. Enzymol. 200:38-62(1991).

[4] Hanks S.K. Curr. Opin. Struct. Biol. 1:369-383(1991).

[5] Hanks S.K., Quinn A.M., Hunter T. Science 241:42-52(1988).

[6] Knighton D.R., Zheng J., Ten Eyck L.F., Ashford V.A., Xuong N.-H., Taylor S.S., Sowadski J.M. Science 253:407-414(1991).

[7] Bairoch A., Claverie J.-M. Nature 331:22(1988).

[8] Benner S. Nature 329:21-21(1987).

[9] Kirby R. J. Mol. Evol. 30:489-492(1992).

[10] Littler E., Stuart A.D., Chee M.S. Nature 358:160-162(1992).

[11] Munoz-Dorado J., Inouye S., Inouye M. Cell 67:995-1006(1991).

Receptor tyrosine kinase class II signature

A number of growth factors stimulate mitogenesis by interacting with a familyof cell surface receptors which possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity [1]. These receptor tyrosine kinases (RTK)all share the same topology: an extracellular ligand-binding domain, a single transmembrane region and a cytoplasmic kinase domain. However they can be classified into at least five groups. The prototype for class II RTK's is the insulin receptor, a heterotetramer of two alpha and two beta chains linked by disulfide bonds. The alpha and beta chains are cleavage products of a precursor molecule. The alpha chain contains the ligand binding site, the beta chain transverses the membrane and contains the tyrosine protein kinase domain. The receptors currently known to belong to class II are: - Insulin receptor from vertebrates. - Insulin growth factor I receptor from mammals. - Insulin receptor-related receptor (IRR), which is most probably a receptor for a peptide belonging to the insulin family. - Insects insulin-like receptors. - Molluscan insulin-related peptide(s) receptor (MIP-R). - Insulin-like peptide receptor from Branchiostoma lanceolatum. - The Drosophila developmental protein sevenless, a putative receptor for positional information required for the formation of the R7 photoreceptor cells. - The trk family of receptors (NTRK1, NTRK2 and NTRK3), which are high affinity receptors for nerve growth factor and related neurotrophic factors (BDNF and NT-3).And the following uncharacterized receptors: - ROS. - LTK (TYK1). - EDDR1 (cak, TRKE, RTK6). - NTRK3 (Tyro10, TKT). - A sponge putative receptor tyrosine kinase. While only the insulin and the insulin growth factor I receptors are known to exist in the tetrameric conformation specific to class II RTK's, all the above proteins share extensive homologies in their kinase domain, especially around the putative site of autophosphorylation. Hence, a signature pattern was developed for this class of RTK's, which includes the tyrosine residue, itself probably autophosphorylated.

Consensus pattern: [DN]-[LIV]-Y-x(3)-Y-Y-R [The second Y is the autophosphorylation site]

[1] Yarden Y., Ullrich A. Annu. Rev. Biochem. 57:443-478(1988).

Receptor tyrosine kinase class III signature

A number of growth factors stimulate mitogenesis by interacting with a family of cell surface receptors which possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity [1]. These receptor tyrosine kinases (RTK)all share the same topology: an extracellular ligand-binding domain, a single transmembrane region and a cytoplasmic kinase domain. However they can be classified into at least five groups. The class III RTK's are characterized by the presence of five to seven immunoglobulin-like domains [2] in their extracellular section. Their kinase domain differs from that of other RTK's by the insertion of a stretch of 70 to 100 hydrophilic residues in the middle ofthis domain. The receptors currently known to belong to class III are: - Platelet-derived growth factor receptor (PDGF-R). PDGF-R exists as a homo- or heterodimer of two related chains: alpha and beta [3]. - Macrophage colony stimulating factor receptor (CSF-1-R) (also known as the fms oncogene). - Stem cell factor (mast cell growth factor) receptor (also known as the kit oncogene). - Vascular endothelial growth factor (VEGF) receptors Flt-1 and Flk-1/KDR [4]. - Fl cytokine receptor Flk-2/Flt-3 [5]. - The putative receptor Flt-4 [7]. a signature pattern Was developed for this class of RTK's which is based on a conserved region in the kinase domain.

Consensus pattern: G-x-H-x-N-[LIVM]-V-N-L-L-G-A-C-T-

[1] Yarden Y., Ullrich A. Annu. Rev. Biochem. 57:443-478(1988).
[2] Hunkapiller T., Hood L. Adv. Immunol. 44:1-63(1989).
[3] Lee K.-H., Bowen-Pope D.F., Reed R.R. Mol. Cell. Biol. 10:2237-2246(1990).

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[4] Terman B.I., Dougher-Vermazen M., Carrion M.E., Dimitrov D., Armellino D.C., Gospodarowicz D., Boehlen P. Biochem. Biophys. Res. Commun. 187:1579-1586(1992).

[5] Lyman S.D., James L., Vanden Bos T., de Vries P., Brasel K., Gliniak B., Hollingsworth L.T., Picha K.S., McKenna H.J., Splett R.R. Cell 75:1157-1167(1993).

[6] Galland F., Karamysheva A., Pebusque M.J., Borg J.P., Rottapel R., Dubreuil P., Rosnet O., Birnbaum D. Oncogene 8:1233-1240(1993).

Receptor tyrosine kinase class V signatures

A number of growth factors stimulate mitogenesis by interacting with a familyof cell surface receptors which possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity [1]. These receptor tyrosine kinases (RTK)all share the same topology: an extracellular ligand-binding domain, a single transmembrane region and a cytoplasmic kinase domain. However they can be classified into at least five groups on the basis of sequence similarities. The extracellular domain of class V RTK's consist of a region of about 300amino acids, amongst which 16 conserved cysteines probably involved in disulfide bonds; this region is followed by two copies of a fibronectin typeIII domain. The ligands for these receptors are proteins of about 200 to 300residues collectively known as Ephrins. The receptors currently known to belong to class V are [2,3,E1]: - EPHA1 (Eph-1; Esk). - EPHA2 (Eck; Mpk-5; Sek-2). - EPHA3 (Etk-1; Hek; Mek4; Tyro4; Rek4; Cek4). - EPHA4 (Sek; Hek8; Mpk-3; Cek8). - EPHA5 (Ehk-1; Hek7; Bsk; Cek7). - EPHA6 (Ehk-2). - EPHA7 (Ehk-3; Hek11; Mdk-1; Ebk). - EPHA8 (Eek). - EPHB1 (Eph-2; Elk; Net). - EPHB2 (Eph-3; Hek5; Drt; Erk; Nuk; Sek-3; Cek5; Qek5). - EPHB3 (Hek-2; Mdk-5). - EPHB4 (Htk; Mdk-2; Myk-1). - EPHB5 (Cek9).The EPHA subtype receptors bind to GPI-anchored ephrins while the EPHB subtype receptors bind to type-I membrane ephrins. Two signature patterns were developed for this class of RTK's, which each include some of the conserved cysteine residues.

Consensus pattern: F-x-[DN]-x-[GAW]-[GA]-C-[LIVM]-[SA]-[LIVM](2)-[SA]-[LV]-[KRHQ]-[LIVA]-x(3)-[KR]-C-[PSAW] [The two C's are probably involved in disulfide bonds]

Consensus pattern: C-x(2)-[DE]-G-[DEQ]-W-x(2,3)-[PAQ]-[LIVMT]-[GT]-x-C-x-C- x(2)-G-[HFY]-[EQ] [The three C's are probably involved in disulfide bonds]

[1] Yarden Y., Ullrich A. Annu. Rev. Biochem. 57:443-478(1988).

[2] Sajjadi F.G., Pasquale E.B., Subramani S. New Biol. 3:769-778(1991).
[3] Wicks I.P., Wilkinson D., Salvaris E., Boyd A.W. Proc. Natl. Acad. Sci. U.S.A. 89:1611-1615(1992).

459. Protein kinase C terminal domain

460. Plant thionins signature

Thionins are small, basic, plant proteins generally toxic to animal cells [1].They seem to exert their toxic effect at the level of the cell membrane but their exact function is not known. They consist of a polypeptide chain of forty five to fifty amino acids with three to four internal disulfide bonds. They are found in seeds but also in the cell wall of leaves [2]. Thionins are processed from larger precursor proteins [3]. Crambin [4], a hydrophobic plant seed protein, also belongs to this family. The pattern to detect this family of proteins includes three of the six cysteine residues involved in disulfide bonds. +----------------------------------+ |+---------------------------+ | || | | xxCCxxxxxxxxxxxCxxxxxxxxxCxxxCxxCxxxxxCxxxxxxxxx ************* | | | +---------+'C': conserved cysteine involved in a disulfide bond.'*': position of the pattern.

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Consensus pattern: C-C-x(5)-R-x(2)-[FY]-x(2)-C [The three C's are involved in disulfide bonds] The proteins from the gamma-thionin family are not related to the above proteins and are described in a separate section.

[1] Vernon L.P., Evett G.E., Zeikus R.D., Gray W.R. Arch. Biochem. Biophys. 238:18-29(1985).
[2] Bohlmann H., Clausen S., Behnke S., Giese H., Hiller C., Reimann-Phillip U., Schrader G., Barkholt V., Apel K. EMBO J. 7:1559-1565(1988).
[3] Bohlmann H., Apel K. Mol. Gen. Genet. 207:446-454(1987).
[4] Teeter M.M., Mazer J.A., L'Italien J.J. Biochemistry 20:5437-5443(1981).

461. Polyprenyl synthetases signatures

A variety of isoprenoid compounds are synthesized by various organisms. For example in eukaryotes the isoprenoid biosynthetic pathway is responsible for the synthesis of a variety of end products including cholesterol, dolichol, ubiquinone or coenzyme Q. In bacteria this pathway leads to the synthesis of isopentenyl tRNA, isoprenoid quinones, and sugar carrier lipids. Among the enzymes that participate in that pathway, are a number of polyprenyl synthetase enzymes which catalyze a 1'4-condensation between 5 carbon isoprene units. Currently the sequence of some of these enzymes is known: - Eukaryotic farnesyl pyrophosphate synthetase (FPP synthetase) (EC 2.5.1.1 / EC 2.5.1.10) which catalyzes the sequential condensation of isopentenyl pyrophosphate (IPP) with dimethylallyl pyrophosphate (DMAPP), and then with the resultant geranyl pyrophosphate to form farnesyl pyrophosphate. FPP synthetase is a cytoplasmic dimeric enzyme. - Prokaryotic farnesyl pyrophosphate synthetase (gene ispA). - Prokaryotic octaprenyl diphosphate synthase (gene ispB). - Prokaryotic heptaprenyl diphosphate synthase (EC 2.5.1.30). - Eukaryotic geranylgeranyl pyrophosphate synthetase (GGPP synthetase) (EC 2.5.1.1 / EC 2.5.1.10 / EC 2.5.1.29) which catalyzes the sequential addition of the three molecules of IPP onto DMAPP to form geranylgeranyl pyrophosphate. In plants GGPP synthase is a chloroplast enzyme involved in the biosynthesis of terpenoids; in fungi, such as Neurospora crassa (gene al-3), this enzyme is involved in the biosynthesis of carotenoids. - Prokaryotic GGPP synthetase, which are involved in the biosynthesis of carotenoids (gene crtE). Such an enzyme is also encoded in the cyanelle genome of Cyanophora paradoxa. - Eukaryotic hexaprenyl pyrophosphate synthetase, which is involved in the biosynthesis of coenzyme Q and which catalyzes the formation of all trans- polyprenyl pyrophosphates generally ranging in length of between 6 and 10 isoprene units depending on the species. HP synthetase is a mitochondrial membrane-associated enzyme. It has been shown [1 to 5] that all the above enzymes share some regions of sequence similarity. Two of these regions are rich in aspartic-acid residues and could be involved in the catalytic mechanism and/or the binding of the substrates. signature patterns were developed for both regions. Possible additional members of this family of proteins are: - Bacillus subtilis spore germination protein C3 (gene gerC3). Both proteins are most probably also enzymes involved in isoprenoid metabolism [6].

Consensus pattern: [LIVM](2)-x-D-D-x(2,4)-D-x(4)-R-R-[GH]-
Consensus pattern: [LIVMFY]-G-x(2)-[FYL]-Q-[LIVM]-x-D-D-[LIVMFY]-x-[DNG]

09555056.092600

[1] Ashby M.N., Edwards P.A. J. Biol. Chem. 265:13157-13164(1990).

[2] Fujisaki S., Hara H., Nishimura Y., Horiuchi K., Nishino T. J. Biochem. 108:995-1000(1990).

[3] Carattoli A., Romano N., Ballario P., Morelli G., Macino G. J. Biol. Chem. 266:5854-5859(1991).

[4] Kuntz M., Roemer S., Suire C., Hugueney P., Weil J.H., Schantz R., Camara B. Plant J. 2:25-34(1992).

[5] Math S.K., Hearst J.E., Poulter C.D. Proc. Natl. Acad. Sci. U.S.A. 89:6761-6764(1992).

[6] Bairoch A. Unpublished observations (1993).

462. Potato inhibitor I family signature

The potato inhibitor I family is one of the numerous families of serine proteinase inhibitors. Members of this protein family are found in plants; in the seeds of barley or beans [1,2,3], and in potato or tomato leaves where they accumulate in response to mechanical damage [4,5]. An inhibitor belonging to this family is also found in leech [6]. It is interesting to note that, currently, this is the only proteinase inhibitor family to be found both inplant and animal kingdoms. Structurally these inhibitors are small (60 to 90 residues) and in contrast with other families of protease inhibitors, they lack disulfide bonds. They have a single inhibitory site. The consensus pattern includes three out of the four residues conserved in all members of this family and is located in the N-terminal half.

Consensus pattern: [FYW]-P-[EQH]-[LIV](2)-G-x(2)-[STAGV]-x(2)-A- Barley subtilisin-chymotrypsin inhibitor-2b has Glu instead of Gly. There is a trypsin inhibitor from the cucurbitaceae Momordica charantia [7], which is said to belong to the potato inhibitor I family but which shows only a very weak similarity with the other members of this family.

[1] Svendsen I., Hejgaard J., Chavan J.K. Carlsberg Res. Commun. 49:493-502(1984).

[2] Svendsen I., Boisen S., Hejgaard J. Carlsberg Res. Commun. 47:45-53(1982).

[3] Nozawa H., Yamagata H., Aizono Y., Yoshikawa M., Iwasaki T. J. Biochem. 106:1003-1008(1989).

[4] Cleveland T.E., Thornburg R.W., Ryan C.A. Plant Mol. Biol. 8:199-207(1987).

[5] Lee J.S., Brown W.E., Graham J.S., Pearce G., Fox E.A., Dreher T.W., Ahern K.G., Pearson G.D., Ryan C.A. Proc. Natl. Acad. Sci. U.S.A. 83:7277-7281(1986).

[6] Seemuller U., Eulitz M., Fritz H., Strobl A. Hoppe-Seyler's Z. Physiol. Chem. 361:1841-1846(1980).

[7] Zeng F.-Y., Qian R.-Q., Wang Y. FEBS Lett. 234:35-38(1988).

463. (pp binding) Phosphopantetheine attachment site

Phosphopantetheine (or pantetheine 4' phosphate) is the prosthetic group of acyl carrier proteins (ACP) in some multienzyme complexes where it serves as a 'swinging arm' for the attachment of activated fatty acid and amino-acid groups [1]. Phosphopantetheine is attached to a serine residue in these proteins [2]. ACP proteins or domains have been found in various enzyme systems which are listed below (references are only provided for recently determined sequences). - Fatty acid synthetase (FAS), which catalyzes the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH. Bacterial and plant chloroplast FAS are composed of eight separate subunits which correspond to the different enzymatic activities; ACP is one of these polypeptides. Fungal FAS consists of two multifunctional proteins, FAS1 and FAS2; the ACP domain is located in the N-terminal section of FAS2. Vertebrate FAS consists of a single multifunctional enzyme; the ACP domain is located between the beta-ketoacyl reductase domain and the C-terminal thioesterase domain [3]. - Polyketide antibiotics synthase enzyme systems. Polyketides are secondary metabolites produced from simple fatty acids, by microorganisms and plants. ACP is one of the polypeptidic components involved in the biosynthesis of Streptomyces polyketide antibiotics actinorhodin, curamycin, granatacin, monensin, oxytetracycline and tetracenomycin C. - Bacillus subtilis putative polyketide synthases pksK, pksL and pksM which respectively contain three, five and one ACP domains. - The multifunctional 6-methysalicylic acid synthase (MSAS) from Penicillium patulum. This is a multifunctional enzyme involved in the biosynthesis of a polyketide antibiotic and which contains an ACP domain in the C-terminal extremity. - Multifunctional mycocerosic acid synthase (gene mas) from Mycobacterium bovis. - Gramicidin S synthetase I (gene grsA) from Bacillus brevis. This enzyme catalyzes the first step in the biosynthesis of the cyclic antibiotic gramicidin S. - Tyrocidine synthetase I (gene tycA) from Bacillus brevis. The reaction carried out by tycA is identical to that catalyzed by grsA - Gramicidin S synthetase II (gene grsB) from Bacillus brevis. This enzyme is a multifunctional protein that activates and polymerizes proline, valine, ornithine and leucine. GrsB contains four ACP domains. - Erythronolide synthase proteins 1, 2 and 3 from Saccharopolyspora erythraea which is involved in the biosynthesis of the polyketide antibiotic erythromicin. Each of these proteins contain two ACP domains. - Conidial green pigment synthase from Aspergillus nidulans. - ACV synthetase from various fungi. This enzyme catalyzes the first step in the biosynthesis of penicillin and cephalosporin. It contains three ACP domains. - Enterobactin synthetase component F (gene entF) from Escherichia coli. This enzyme is involved in the ATP-dependent activation of serine during enterobactin (enterochelin) biosynthesis. - Cyclic peptide antibiotic surfactin synthase subunits 1, 2 and 3 from Bacillus subtilis. Subunits 1 and 2 contains three related domains while subunit 3 only contains a single domain. - HC-toxin synthetase (gene HTS1) from Cochliobolus carbonum. This enzyme synthesizes HC-toxin, a cyclic tetrapeptide. HTS1 contains four ACP domains. - Fungal mitochondrial ACP [9], which is part of the respiratory chain NADH dehydrogenase (complex I). - Rhizobium nodulation protein nodF, which probably acts as an ACP in the synthesis of the nodulation Nod factor fatty acyl chain.The sequence around the phosphopantetheine attachment site is conserved in all these proteins and can be used as a signature pattern. A profile was also developed that spans the complete ACP-like domain.

Consensus pattern: [DEQGSTALMKRH]-[LIVMFYSTAC]-[GNQ]-[LIVMFYAG]-[DNEKHS]-S- [LIVMST]-{PCFY}-[STAGCPQLIVMF]-[LIVMATN]-[DENQGTAKRHLM]- [LIVMWSTA]-[LIVGSTACR]-x(2)-[LIVMFA] [S is the pantetheine attachment site]

[1] Concise Encyclopedia Biochemistry, Second Edition, Walter de Gruyter, Berlin New-York (1988).

[2] Pugh E.L., Wakil S.J. J. Biol. Chem. 240:4727-4733(1965).

[3] Witkowski A., Rangan V.S., Randhawa Z.I., Amy C.M., Smith S. Eur. J. Biochem. 198:571-579(1991).

[6] Scotti C., Piatti M., Cuzzoni A., Perani P., Tognoni A., Grandi G., Galizzi A., Albertini A.M. Gene 130:65-71(1993).

[9] Sackmann U., Zensen R., Rohlen D., Jahnke U., Weiss H. Eur. J. Biochem. 200:463-469(1991).

464. (Prenyltrans) Terpene synthases signature

The following enzymes catalyze mechanistically related reactions which involvethe highly complex cyclic rearrangement of squalene or its 2,3 oxide: - Lanosterol synthase (EC 5.4.99.7) (oxidosqualene--lanosterol cyclase), which catalyzes the cyclization of (S)-2,3-epoxysqualene to lanosterol, the initial precursor of cholesterol, steroid hormones and vitamin D in vertebrates and of ergosterol in fungi (gene ERG7). - Cycloartenol synthase (EC 5.4.99.8) (2,3-epoxysqualene--cycloartenol cyclase), a plant enzyme that catalyzes the cyclization of (S)-2,3- epoxysqualene to cycloartenol. - Hopene synthase (EC 5.4.99.-) (squalene--hopene cyclase), a bacterial enzyme that catalyzes the cyclization of squalene into hopene, a key step in hopanoid (triterpenoid) metabolism.These enzymes are evolutionary related [1] proteins of about 70 to 85 Kd. As a signature pattern, a highly conserved region was selected which is rich in aromatic residues and which is located in the C-terminal section.

Consensus pattern: [DE]-G-S-W-x-G-x-W-[GA]-[LIVM]-x-[FY]-x-Y-[GA]

[1] Corey E.J., Matsuda S.P.T., Bartel B. Proc. Natl. Acad. Sci. U.S.A. 90:11628-11632(1993).

465. Prion protein signatures

Prion protein (PrP) [1,2,3] is a small glycoprotein found in high quantity in the brains of humans or animals infected with a number of degenerative neurological diseases such as Kuru, Creutzfeldt-Jacob disease (CJD), scrapie or bovine spongiform encephalopathy (BSE). PrP is encoded in the host genome and expressed both in normal and infected cells. It has a tendency to aggregate yielding polymers called rods. Structurally, PrP is a protein consisting of a signal peptide, followed by an N-terminal domain that contains tandem repeats of a short motif (PHGGGWGQin mammals, PHNPGY in chicken), itself followed by a highly conserved domain lly comes a C-terminal hydrophobic domain post-translationally removed when PrP is attachedto the extracellular side of the cell membrane by a GPI-anchor. The structureof PrP is shown in the following schematic representation: +---+----------------+-******-------------------****-----+-----+ |Sig| Tandem repeats | C C S| | +---+----------------+---------------------|--------|----|+-----+ +--------+ | GPI'C': conserved cysteine involved in a disulfide bond.'*': position of the patterns. As signature pattern for PrP, a perfectly conserved alanine- and glycine-rich region of 16 residues was selected as well as a region centered on the second cysteine involved in the disulfide bond.

Consensus pattern: A-G-A-A-A-A-G-A-V-V-G-G-L-G-G-Y-
Consensus pattern: E-x-[ED]-x-K-[LIVM](2)-x-[KR]-[LIVM](2)-x-[QE]-M-C-x(2)- Q-Y [C is involved in a disulfide bond]

[1] Stahl N., Prusiner S.B. FASEB J. 5:2799-2807(1991).
[2] Brunori M., Chiara Silvestrini M., Pocchiari M. Trends Biochem. Sci. 13:309-313(1988).
[3] Prusiner S.B. Annu. Rev. Microbiol. 43:345-374(1989).

466. Cyclophilin-type peptidyl-prolyl cis-trans isomerase signature and profile (pro isomerase)

Cyclophilin [1] is the major high-affinity binding protein in vertebrates for the immunosuppressive drug cyclosporin A (CSA). It exhibits a peptidyl- prolyl cis-trans isomerase activity (EC 5.2.1.8) (PPIase or rotamase). PPIase is an enzyme that accelerates protein folding by catalyzing the cis-transisomerization of proline imidic peptide bonds in oligopeptides [2]. It is probable that CSA mediates some of its effects via an inhibitory action on PPIase. Cyclophilin is a cytosolic protein which belongs to a family [3,4,5]that also includes the following isozymes: - Cyclophilin B (or S-cyclophilin), a PPIase which is retained in an endoplasmic reticulum compartment. - Cyclophilin C, a cytoplasmic PPiase. - Mitochondrial matrix cyclophilin (cyp3). - A PPIase which seems specific for the folding of rhodopsin and is an integral membrane protein anchored by a C-terminal transmembrane region. This protein was first characterized in Drosophila (gene ninaA). - Bacterial periplasmic PPiase (gene ppiA). - Bacterial cytosolic PPiase (gene ppiB). - Natural-killer cell cyclophilin-related protein. This large protein (about 160 Kd) is a component of a putative tumor-recognition complex involved in the function of NK cells. It contains a cyclophilin-type PPiase domain. - Mammalian nucleoporin Nup358 [6], a nuclear pore complex protein of 358 Kd that contains a C-terminal cyclophilin-type PPiase domain. - Yeast hypothetical protein YJR032w. - Fission yeast hypothetical protein SpAC21E11.05c. - Caenorhabditis elegans hypothetical protein T27D1.1.The sequences of the different forms of cyclophilin-type PPIases are well conserved. As a signature pattern, a conserved region was selected in the central part of these enzymes.

Consensus pattern: [FY]-x(2)-[STCNLV]-x-F-H-[RH]-[LIVMN]-[LIVM]-x(2)-F- [LIVM]-x-Q-[AG]-G- FKBP's, a family of proteins that bind the immunosuppressive drug FK506, are also PPIases, but their sequence is not at all related to that of cyclophilin.

[1] Stamnes M.A., Rutherford S.L., Zuker C.S. Trends Cell Biol. 2:272-276(1992).
[2] Fischer G., Schmid F.X. Biochemistry 29:2205-2212(1990).
[3] Trandinh C.C., Pao G.M., Saier M.H. Jr. FASEB J. 6:3410-3420(1992).
[4] Galat A. Eur. J. Biochem. 216:689-707(1993).
[5] Hacker J., Fischer G. Mol. Microbiol. 10:445456(1993).
[6] Wu J., Matunis M.J., Kraemer D., Blobel G., Coutavas E. J. Biol. Chem. 270:14209-14213(1995).

467. Profilin signature

Profilin [1,2] is a small eukaryotic protein that binds to monomeric actin(G-actin) in a 1:1 ratio thus preventing the polymerization of actin into filaments (F-actin). It can also, in certain circumstance promotes actin polymerization. Profilin also binds to polyphosphoinositides such as PIP2.Overall sequence similarity among profilin from organisms which belong to different phyla (ranging from fungi to mammals) is low, but the N-terminal region is relatively well conserved. That region is thought to be involved inthe binding to actin. The signature pattern for profilin is based on conserved residues at the N-terminal extremity .A protein structurally similar to profilin is present in the genome of variola and vaccinia viruses (gene A42R).

Consensus pattern: <x(0,1)-[STA]-x(0,1)-W-[DENQH]-x-[YI]-x-[DEQ]

[1] Haarer B.K., Brown S.S. Cell Motil. Cytoskeleton 17:71-74(1990).
[2] Sohn R.H., Goldschmidt-Clermont P. BioEssays 16:465-472(1994).

468. Protamine P1 signature

Protamines are small, highly basic proteins, that substitute for histones in sperm chromatin during the haploid phase of spermatogenesis. They pack sperm DNA into a highly condensed, stable and inactive complex. There are two different types of mammalian protamine, called P1 and P2. P1 has been found in all species studied, while P2 is sometimes absent. There seems to be a single type of avian protamine whose sequence is closely related to that of mammalian P1 [1].As a signature for this family of proteins, a conserved region was selected at the N-terminal extremity of the sequence.

Consensus pattern: [AV]-R-[NFY]-R-x(2,3)-[ST]-x-S-x-S-

[1] Oliva R., Goren R., Dixon G.H. J. Biol. Chem. 264:17627-17630(1989).

469. Sperm histone P2 (protamine P2)

This protein also known as protamine P2 can substitute for histones in the chromatin of sperm. The alignment contains both the sequence of the mature P2 protein and its propeptide.

470. Proteasome A-type subunits signature

The proteasome (or macropain) (EC 3.4.99.46) [1 to 5,E1] is an eukaryotic and archaebacterial multicatalytic proteinase complex that seems to be involved inan ATP/ubiquitin-dependent nonlysosomal proteolytic pathway. In eukaryotes the proteasome is composed of about 28 distinct subunits which form a highly ordered ring-shaped structure (20S ring) of about 700 Kd. Most proteasome subunits can be classified, on the basis on sequence similarities into two groups, A and B. Subunits that belong to the A-type group are proteins of from 210 to 290 amino acids that share a number of conserved sequence regions. Subunits that are known to belong to this family are listed below. - Vertebrate subunits C2 (nu), C3, C8, C9, iota and zeta. - Drosophila PROS-25, PROS-28.1, PROS-29 and PROS-35. - Yeast C1 (PRS1), C5 (PRS3), C7-alpha (Y8) (PRS2), Y7, Y13, PRE5, PRE6 and PUP2. - Arabidopsis thaliana subunits alpha and PSM30. - Thermoplasma acidophilum alpha-subunit. In this archaebacteria the proteasome is composed of only two different subunits.As a signature pattern for proteasome A-type subunits the best conserved region was selected, which is located in the N-terminal part of these proteins.

Consensus pattern: [FY]-x(4)-[STNV]-x-[FYW]-S-P-x-G-[RKH]-x(2)-Q-[LIVM]-[DE]- Y-[SAD]-x(2)-[SAG]-. These proteins belong to family T1 in the classification of peptidases [6,E2].

[1] Rivett A.J. Biochem. J. 291:1-10(1993).
[2] Rivett A.J. Arch. Biochem. Biophys. 268:1-8(1989).
[3] Goldberg A.L., Rock K.L Nature 357:375-379(1992).
[4] Wilk S. Enzyme Protein 47:187-188(1993).
[5] Hilt W., Wolf D.H. Trends Biochem. Sci. 21:96-102(1996).
[6] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

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Proteasome B-type subunits signature

The proteasome (or macropain) (EC 3.4.99.46) [1 to 5,E1] is an eukaryotic and archaebacterial multicatalytic proteinase complex that seems to be involved in an ATP/ubiquitin-dependent nonlysosomal proteolytic pathway. In eukaryotes the proteasome is composed of about 28 distinct subunits which form a highly ordered ring-shaped structure (20S ring) of about 700 Kd. Most proteasome subunits can be classified, on the basis on sequence similarities into two groups, A and B. Subunits that belong to the B-type group are proteins of from 190 to 290 amino acids that share a number of conserved sequence regions. Subunits that are known to belong to this family are listed below. - Vertebrate subunits C5, beta, delta, epsilon, theta (C10-II), LMP2/RING12, C13 (LMP7/RING10), C7-I and MECL-1. - Yeast PRE1, PRE2 (PRG1), PRE3, PRE4, PRS3, PUP1 and PUP3. - Drosophila L(3)73AI. - Fission yeast pts1. - Thermoplasma acidophilum beta-subunit. In this archaebacteria the proteasome is composed of only two different subunits. As a signature pattern for proteasome B-type subunits the best conserved region was selected, which is located in the N-terminal part of these proteins.

Consensus pattern: [LIVMA]-[GSA]-[LIVMF]-x-[FYLVGAC]-x(2)-[GSACFY]-[LIVMSTAC](3)-[GAC]-[GSTACV]-[DES]-x(15)-[RK]-x(12,13)-G-x(2)-[GSTA]-D-. These proteins belong to family T1 in the classification of peptidases [6,E2].

[1] Rivett A.J. Biochem. J. 291:1-10(1993).
[2] Rivett A.J. Arch. Biochem. Biophys. 268:1-8(1989).
[3] Goldberg A.L., Rock K.L Nature 357:375-379(1992).
[4] Wilk S. Enzyme Protein 47:187-188(1993).
[5] Hilt W., Wolf D.H. Trends Biochem. Sci. 21:96-102(1996).
[6] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

471. (pyr redox) Pyridine nucleotide-disulphide oxidoreductases class-I active site

The pyridine nucleotide-disulphide oxidoreductases are FAD flavoproteins which contains a pair of redox-active cysteines involved in the transfer of reducing equivalents from the FAD cofactor to the substrate. On the basis of sequence and structural similarities [1] these enzymes can be classified into two categories. The first category groups together the following enzymes [2 to 6]: - Glutathione reductase (EC 1.6.4.2) (GR). - Higher eukaryotes thioredoxin reductase (EC 1.6.4.5). - Trypanothione reductase (EC 1.6.4.8). - Lipoamide dehydrogenase (EC 1.8.1.4), the E3 component of alpha-ketoacid dehydrogenase complexes. - Mercuric reductase (EC 1.16.1.1).The sequence around the two cysteines involved in the redox-active disulfide bond is conserved and can be used as a signature pattern.

Consensus pattern: G-G-x-C-[LIVA]-x(2)-G-C-[LIVM]-P [The two C's form the active site disulfide bond]. In positions 6 and 7 of the pattern all known sequences have Asn-(Val/ Ile) with the exception of GR from plant chloroplasts and from cyanobacteria which have Ile-Arg [7].

[1] Kurlyan J., Krishna T.S.R., Wong L., Guenther B., Pahler A., Williams C.H. Jr., Model P. Nature 352:172-174(1991).
[2] Rice D.W., Schulz G.E., Guest J.R. J. Mol. Biol. 174:483-496(1984).
[3] Brown N.L. Trends Biochem. Sci. 10:400-402(1985).
[4] Carothers D.J., Pons G., Patel M.S. Arch. Biochem. Biophys. 268:409-425(1989).
[5] Walsh C.T., Bradley M., Nadeau K. Trends Biochem. Sci. 16:305-309(1991).
[6] Gasdaska P.Y., Gasdaska J.R., Cochran S., Powis G. FEBS Lett. 373:5-9(1995).

[7] Creissen G., Edwards E.A., Enard C., Wellburn A., Mullineaux P. Plant J. 2:129-131(1991).

472. (pyridoxal deC) DDC / GAD / HDC / TyrDC pyridoxal-phosphate attachment site (pyridoxal deC)

Three different enzymes - all pyridoxal-dependent decarboxylases - seem to share regions of sequence similarity [1,2,3,4], especially in the vicinity of the lysine residue which serves as the attachment site for the pyridoxal-phosphate (PLP) group. These enzymes are: - Glutamate decarboxylase (EC 4.1.1.15) (GAD). Catalyzes the decarboxylation of glutamate into the neurotransmitter GABA (4-aminobutanoate). - Histidine decarboxylase (EC 4.1.1.22) (HDC). Catalyzes the decarboxylation of histidine to histamine. There are two completely unrelated types of HDC: those that use PLP as a cofactor (found in Gram-negative bacteria and mammals), and those that contain a covalently bound pyruvoyl residue (found in Gram-positive bacteria). - Aromatic-L-amino-acid decarboxylase (EC 4.1.1.28) (DDC), also known as L-dopa decarboxylase or tryptophan decarboxylase. DDC catalyzes the decarboxylation of tryptophan to tryptamine. It also acts on 5-hydroxy- tryptophan and dihydroxyphenylalanine (L-dopa). - Tyrosine decarboxylase (EC 4.1.1.25) (TyrDC) which converts tyrosine into tyramine, a precursor of isoquinoline alkaloids and various amides.These enzymes are collectively known as group II decarboxylases [3,4].

Consensus pattern: S-[LIVMFYW]-x(5)-K-[LIVMFYWG](2)-x(3)-[LIVMFYW]-x-[CA]-x(2)-[LIVMFYWQ]-x(2)-[RK] [K is the pyridoxal-P attachment site]

[1] Jackson F.R. J. Mol. Evol. 31:325-329(1990).
[2] Joseph D.R., Sullivan P., Wang Y.-M., Kozak C., Fenstermacher D.A., Behrendsen M.E., Zahnow C.A. Proc. Natl. Acad. Sci. U.S.A. 87:733-737(1990).
[3] Sandmeier E., Hale T.I., Christen P. Eur. J. Biochem. 221:997-1002(1994).
[4] Ishii S., Mizugichi H., Nishino J., Hayashi H., Kagamiyama H. J. Biochem. 120:369-376(1996).

473. Regulator of chromosome condensation (RCC1) signatures (RCC1)

The regulator of chromosome condensation (RCC1) [1] is a eukaryotic protein which binds to chromatin and interacts with ran, a nuclear GTP-binding protein, to promote the loss of bound GDP and the uptake offresh GTP, thus acting as a guanine-nucleotide dissociation stimulator (GDS)[2]. The interaction of RCC1 with ran probably plays an important role in the regulation of gene expression. RCC1, known as PRP20 or SRM1 in yeast, pim1 in fission yeast and BJ1 in Drosophila, is a protein that contains seven tandem repeats of a domain of about 50 to 60 amino acids. As shown in the following schematic representation, the repeats make up the major part of the length of the protein. Outside the repeat region, there is just a small N-terminal domain of about 40 to 50 residues and, in the Drosophila protein only, a C-terminal domain of about 130 residues. +----+-------+-------+-------+-------+-------+-------+-------+-------------+ |N-t.|Rpt. 1 |Rpt. 2 |Rpt. 3 |Rpt. 4 |Rpt. 5 |Rpt. 6 |Rpt. 7 | C-terminal | +----+-------+-------+-------+-------+-------+-------+-------+-------------+ In Drosophila two signature patterns for RCC1 were developed. The first is found in the N- terminal part of the second repeat; this is the most conserved part of RCC1. The second is derived from conserved positions in the C-terminal part of each repeat and detects up to five copies of the repeated domain. The RCC1-type of repeat is also found in the X-linked retinitis pigmentosa GTPase regulator [3].

Consensus pattern: G-x-N-D-x(2)-[AV]-L-G-R-x-T-
Consensus pattern: [LIVMFA]-[STAGC](2)-G-x(2)-H-[STAGLI]-[LIVMFA]-x-[LIVM]-

[1] Dasso M. Trends Biochem. Sci. 18:96-101(1993).
[2] Boguski M.S., McCormick F. Nature 366:643-654(1993).
[3] Roepman R., Van Duijnhoven G., Rosenberg T., Pinckers A.J.L.G., Bleeker-Wagemakers L.M., Bergen A.A.B., Post J., Beck A., Reinhardt R., Ropers H.-H., Cremers F., Berger W. Hum. Mol. Genet. 5:1035-1041(1996).

474. RNA 3'-terminal phosphate cyclase signature (RCT)

RNA 3'-terminal phosphate cyclase (EC 6.5.1.4) [1,2] catalyzes the conversion of 3'-phosphate to a 2',3'-cyclic phosphodiester at the end of RNA. The biological role of this enzyme is unknown but it is likely to function in some aspects of cellular RNA processing. The reaction catalyzed by the enzyme occurs in three steps: 1) adenylation of the enzyme by

ATP; 2) the enzyme acts on RNA-3'terminal phosphate to produce RNA-3'terminal diphosphate adenylate; 3) Release of AMP and cyclisation by a non catalytic nucleophilic attack by the adjacent 2'hydroxyl on the phosphorus in the diester linkage. This enzyme, which has been characterized in human (where there seems to be at least three isozymes) and Escherichia coli (gene rtCA), seems to be taxonomically widespread. It is found in insects, plants, fungi (gene RTC1 inyeast) and in archeabacteria. RNA cyclase is a protein of from 36 to 42 Kd. The best conserved region, which is used as a signature pattern, is a glycine-rich stretch of residues located in the central part of the sequence and which is reminiscent of various ATP, GTPor AMP glycine-rich loops. In this context, the conserved Arg (His in the E.coli enzyme) could be the AMP-binding residue.

Consensus pattern: [RH]-G-x(2)-P-x-G(3)-x-[LIV]-

[1] Genschik P., Billy E., Swianiewicz M., Filipowicz W. EMBO J. 16:2955-2967(1997).
[2] Filipowicz W., Vincente O. Meth. Enzymol. 181:499-510(1990).

475. REV protein (anti-repression trans-activator protein)

476. Prokaryotic-type class I peptide chain release factors signature (RF-1)

Peptide chain release factors (RFs) are required for the termination of protein biosynthesis [1]. At present two classes of RFs can be distinguished. Class I RFs bind to ribosomes that have encountered a stop codon at their decoding site and induce release of the nascent polypeptide. Class II RFs are GTP-binding proteins that interact with class I RFs and enhance class I RF activity. In prokaryotes there are two class I RFs that act in a codon specific manner[2]: RF-1 (gene prfA) mediates UAA and UAG-dependent termination while RF-2(gene prfB) mediates UAA and UGA-dependent termination. RF-1 and RF-2 are structurally and evolutionary related proteins which have been shown [3] to make up a family that also contains the following proteins: - Fungal MRF1, a mitochondrial RF (m-RF) which recognizes the UAA and UAG codons. - Escherichia coli RF-H, a protein of unknown function. - Escherichia coli hypothetical protein yaeJ and a close Pseudomonas putida homolog. A highly conserved region located in the central part of the 40 to 45 Kd RF-1/2 and m-RF and in the N-terminal of the 15 to 16Kd RF-H and yaeJ is used as a signature pattern.

Consensus pattern: [AR]-[STA]-x-G-x-G-G-Q-[HNGCS]-V-N-x(3)-[ST]-A-[IV]
Note that prokaryotic-type class I RFs display no significant sequence similarity to prokaryotic-type class II which belong to the family of GTP-binding elongation factors nor to eukaryotic class I or class II RFs.

[1] Tate W.P. , Poole E.S., Mannering S.M. Prog. Nucleic Acids. Res. Mol. Biol. 52:293-335(1996).
[2] Craigen W.J., Lee C.C., Caskey C.T. Mol. Microbiol. 4:861-865(1990).
[3] Pel H.J., Rep M., Grivell L.A. Nucleic Acids Res. 20:4423-4428(1992).

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477. RIO1/ZK632.3/MJ0444 family signature
The following uncharacterized proteins are evolutionary related [1]: - Yeast protein RIO1. - Caenorhabditis elegans hypothetical protein ZK632.3. - Methanococcus jannaschii hypothetical protein MJ0444. - Thermoplasma acidophilum hypothetical protein if rpoA2 3'region.The eukaryotic members of this family are proteins of about 55 to 60 Kd, while the archebacterial ones are half that size. The central part of these proteins is highly conserved. The best conserved region is used as a signature pattern.

Consensus pattern: [LIVM]-V-H-[GA]-D-L-S-E-[FY]-N-x-[LIVM]

[1] Bairoch A. Unpublished observations (1997).

478. (RIP) Shiga/ricin ribosomal inactivating toxins active site signature. A number of bacterial and plant toxins act by inhibiting protein synthesis in eukaryotic cells. The toxins of the Shiga and ricin family inactivate 60S ribosomal subunits by an N-glycosidic cleavage which releases a specific adenine base from the sugar-phosphate backbone of 28S rRNA [1,2,3]. The toxins which are known to function in this manner are: - Shiga toxin from Shigella dysenteriae [4]. This toxin is composed of one copy of an enzymatically active A

subunit and five copies of a B subunit responsible for binding the toxin complex to specific receptors on the target cell surface. - Shiga-like toxins (SLT) are a group of Escherichia coli toxins very similar in their structure and properties to Shiga toxin. The sequence of two types of these toxins, SLT-1 [5] and SLT-2 [6], is known. - Ricin, a potent toxin from castor bean seeds. Ricin consists of two glycosylated chains linked by a disulfide bond. The A chain is enzymatically active. The B chain is a lectin with a binding preference for galactosides. Both chains are encoded by a single polypeptidic precursor. Ricin is classified as a type-II ribosome-inactivating protein (RIP); other members of this family are agglutinin, also from castor bean, and abrin from the seeds of the bean Abrus precatorius [7]. - Single chain ribosome-inactivating proteins (type-I RIP) from plants. Examples of such proteins are: barley protein synthesis inhibitors I and II, mongolian snake-gourd trichosanthin, sponge gourd luffin-A and -B, garden four-o'clock MAP, common pokeberry PAP-S and soapwort saporin-6 [7].All these toxins are structurally related. A conserved glutamic residue has been implicated [8] in the catalytic mechanism; it is located near a conserved arginine which also plays a role in catalysis [9]. The signature that has been developed for these proteins includes these catalytic residues.

Consensus pattern: [LIVMA]-x-[LIVMSTA](2)-x-E-[SAGV]-[STAL]-R-[FY]-[RKNQS]-x-[LIVM]-[EQS]-x(2)-[LIVMF] [E and R are active site residues]-

[1] Endo Y., Tsurugi K., Takeda Y., Ogasawara T., Igarashi K. Eur. J. Biochem. 171:45-50(1988).[2] May M.J., Hartley M.R., Roberts L.M., Krieg P.A., Osborn R.W., Lord J.M. EMBO J. 8:301-308(1989).[3] Funatsu G., Islam M.R., Minami Y., Sung-Sil K., Kimura M. Biochimie 73:1157-1161(1991).[4] Strockbine N.A., Jackson M.P., Sung L.M., Holmes R.K., O'Brien A.D. J. Bacteriol. 170:1116-1122(1988).[5] Calderwood S.B., Auclair F., Donohue-Rolfe A., Keusch G.T., Mekalanos J.J. Proc. Natl. Acad. Sci. U.S.A. 84:4364-4368(1987).[6] Jackson M.P., Neill R.J., O'Brien A.D., Holmes R.K., Newland J.W. FEMS Microbiol. Lett. 44:109-114(1987).[7] Barbieri L., Battelli M.G., Stirpe F. Biochim. Biophys. Acta 1154:237-282(1993).[8] Hovde C.J., Calderwood S.B., Mekalanos J.J., Collier R.J. Proc. Natl. Acad. Sci. U.S.A. 85:2568-2572(1988).[9] Monzingo A.F., Collins E.J., Ernst S.R., Irvin J.D., Robertus J.D. J. Mol. Biol. 233:705-715(1993).

09525325.061500

479. Bacterial RNA polymerase, alpha chain (RNA pol A bac)

Members of this family include alpha subunit from eubacteria and alpha subunits from chloroplasts. The alpha subunit of RNA polymerase consists of two independently folded domains, referred to as amino-terminal and carboxyl terminal domains. The amino terminal domain is involved in the interaction with the other subunits of the RNA polymerase. The carboxyl-terminal domain interacts with the DNA and activators. The amino acid sequence of the alpha subunit is conserved in prokaryotic and chloroplast RNA polymerases. There are three regions of particularly strong conservation, two in the amino-terminal and one in the carboxyl-Comment: terminal [3].

[1] Zhang G, Darst SA; Science 1998;281:262-266. [2] Jeon YH, Negishi T, Shirakawa M, Yamazaki T, Fujita N, Ishihama A, Kyogoku Y; Science 1995;270:1495-1497. [3] Ebright RH, Busby S; Curr Opin Genet Dev 1995;5:197-203. [4] Murakami K, Kimura M, Owens JT, Meares CF, Ishihama A; Proc Natl Acad Sci USA 1997;94:1709-1714.

09565305.052601

480. RNA polymerase beta subunit (RNA pol B)

RNA polymerases catalyse the DNA dependent polymerisation of RNA. Prokaryotes contain a single RNA polymerase compared to three in eukaryotes (not including mitochondrial and chloroplast polymerases). Each RNA polymerase complex contains two related members of this family, in each case they are the two largest subunits.

[1] Falkenburg D, Dworniczak B, Faust DM, Bautz EK; J Mol Biol 1987;195:929-937.

481. RNA polymerases H / 23 Kd subunits signature

In eukaryotes, there are three different forms of DNA-dependent RNA polymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaebacteria, there is generally a single form of RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides. Archaebacterial subunit H (gene rpoH) [1,2] is a small protein of about 8.5 to10 Kd, it is evolutionary related to the C-terminal part of a 23 Kd component shared by all three forms of eukaryotic RNA polymerases (gene RPB5 in yeast and POLR2E in mammals).As a signature pattern a conserved region was selected which is located at theN-terminal extremity of subunit H; this region contains two histidines that could play a role in the binding of a metal ion.

Consensus pattern: H-[NEI]-[LIVM]-V-P-x-H-x(2)-[LIVM]-x(2)-[DE]

[1] Klenk H.-P., Palm P., Lottspeich F., Zillig W. Proc. Natl. Acad. Sci. U.S.A. 89:407-410(1992).
[2] Thiru A., Hodach M., Eloranta J.J., Kostourou V., Weinzierl R.O., Matthews S.; J. Mol. Biol. 287:753-760(1999).

09585253.061502

482. RNA polymerases K / 14 to 18 Kd subunits signature

In eukaryotes, there are three different forms of DNA-dependent RNApolymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaebacteria, there is generally a single form of RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides. A component of 14 to 18 Kd shared by all three forms of eukaryotic RNA polymerases and which has been sequenced in budding yeast (gene RPB6 orRPO26), in fission yeast (gene rpb6 or rpo15), in human and in African swine fever virus [1] is evolutionary related [2] to archaebacterial subunit K (gene rpoK). The archaebacterial protein is colinear with the C-terminal part of the eukaryotic subunit.

Consensus pattern: [ST]-x-[FY]-E-x-[AT]-R-x-[LIVM]-[GSA]-x-R-[SA]-x-Q

[1] Lu Z., Kutish G.F., Sussman M.D., Rock D.L. Nucleic Acids Res. 21:2940-2940(1993).
[2] McKune K., Woychik N.A. J. Bacteriol. 176:4754-4756(1994).

483. RNA polymerases L / 13 to 16 Kd subunits signature

In eukaryotes, there are three different forms of DNA-dependent RNApolymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaebacteria, there is generally a single form of RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides. It has been shown that small subunits of about 13 to 16 Kd found in all three types of eukaryotic polymerases are highly conserved. Subunits known to belong to this family are: - Budding yeast RPC19 subunit from RNA polymerases I and III [1]. - Budding yeast RPB11 subunit from RNA polymerase II [2]. - Mammalian RPB11 (gene POLR2K) from RNA polymerase II. - Caenorhabditis elegans hypothetical protein F58A4.9. - Methanococcus jannaschii RNA polymerase subunit L (gene rpoL). - Sulfolobus acidocaldarius RNA polymerase subunit L (gene rpoL) [3].As a signature pattern a conserved region was selected which is located at the N-terminal extremity of these polymerase subunits; this region contains two cysteines that could play a role in the binding of a metal ion.

Consensus pattern: [DE](2)-H-[ST]-[LIVM]-[GAP]-N-x(11)-V-x-[FM]-x(2)-Y-x(3)- H-P

[1] Dequard-Chablat M., Riva M., Carles C., Sentenac A. J. Biol. Chem. 266:15300-15307(1991).

[2] Woychik N.A., McKune K., Lane W.S., Young R.A. Gene Expr. 3:77-82(1993).

[3] Langer D. EMBL/GenBank: X70805.

09555350.051502

484. RNA polymerases N / 8 Kd subunits signature

In eukaryotes, there are three different forms of DNA-dependent RNA polymerases (EC 2.7.7.6) transcribing different sets of genes. Each class of RNA polymerase is an assemblage of ten to twelve different polypeptides. In archaebacteria, there is generally a single form of RNA polymerase which also consist of an oligomeric assemblage of 10 to 13 polypeptides. Archaebacterial subunit N (gene rpoN) [1] is a small protein of about 8 Kd, it is evolutionary related [2] to a 8.3 Kd component shared by all three forms of eukaryotic RNA polymerases (gene RPB10 in yeast and POLR2J in mammals) as well as to African swine fever virus protein CP80R [3].As a signature pattern a conserved region was selected which is located at the N-terminal extremity of these polymerase subunits; this region contains two cysteines that could play a role in the binding of a metal ion.

Consensus pattern: [LIVMF](2)-P-[LIVM]-x-C-F-[ST]-C-G-

[1] Langer D., Hain J., Thuriaux P., Zillig W. Proc. Natl. Acad. Sci. U.S.A. 92:5768-5772(1995).

[2] McKune K., Woychik N.A. J. Bacteriol. 176:4754-4756(1994).

[3] Yanez R.J., Rodriguez J.M., Nogal M.L., Yuste L., Enriquez C., Rodriguez J.F., Vinuela E. Virology 208:249-278(1995).

485. Ribonuclease HII

[1] Mian IS; Nucleic Acids Res 1997;25:3187-3189.

486. Ribonuclease PH signature

Prokaryotic ribonuclease PH (EC 2.7.7.56) (RNase PH) [1] is a phosphorolyticexoribonuclease that removes nucleotide residues following the -CCA terminus of tRNA and adds nucleotides to the ends of RNA molecules by using nucleoside diphosphates as substrates. RNase PH is a conserved protein of about 240 amino-acid residues. It is evolutionary related to Caenorhabditis elegans hypothetical protein B0564.1.As a signature pattern, the most highly conserved region was selected which is located in the central part of these proteins.

Consensus sequence: C-[DE]-[LIVM](2)-Q-[GTA]-D-G-[SG]-x(2)-[TA]-A

[1] Kelly K.O., Deutscher M.P. J. Biol. Chem. 267:17153-17158(1992).

487. RanBP1 domain

[1] Di Matteo G, Fuschi P, Zerfass K, Moretti S, Ricordy R, Cenciarelli C, Tripodi M, Jansen-Durr P, Lavia P; Cell Growth Differ 1995;6:1213-1224.

488. Rhodanese signatures

Rhodanese (thiosulfate sulfurtransferase) (EC 2.8.1.1) [1,2] is an enzyme which catalyzes the transfer of the sulfane atom of thiosulfate to cyanide, to form sulfite and thiocyanate. In vertebrates, rhodanese is a mitochondrial enzyme of about 300 amino-acid residues involved in forming iron-sulfur complexes and cyanide detoxification. A cysteine residue takes part in the catalytic mechanism. Some bacterial proteins closely related to rhodanese are also thought to express a sulfotransferase activity. These are: - Azotobacter vinelandii rhdA. - Escherichia coli sseA [3]. - Saccharopolyspora erythraea cysA [4]. - Synechococcus strain PCC 7942 rhdA [5]. RhdA is a periplasmic protein probably involved in the transport of sulfur compounds. Two patterns for the rhodanese family were developed. They are based on highly conserved regions, one which is located in the N-terminal region, the other at the C-terminal extremity of the enzyme.

Consensus pattern: [FY]-x(3)-H-[LIV]-P-G-A-x(2)-[LIVF]
Consensus pattern: [FY]-[DEAP]-G-[SA]-W-x-E-[FYW]

[1] Westley J. Meth. Enzymol. 77:285-291(1981).
[2] Weiland K.L., Dooley T.P. Biochem. J. 275:227-231(1991).
[3] Rudd K.E. Unpublished observations (1993).
[4] Donadio S., Shafiee A., Hutchinson C.R. J. Bacteriol. 172:350-360(1990).
[5] Laudenbach D.E., Ehrhardt D., Green L., Grossman A.R. J. Bacteriol. 173:2751-2760(1991).

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489. Ribonuclease III family signature

Prokaryotic ribonuclease III (EC 3.1.26.3) (gene rnc) [1] is an enzyme that digests double-stranded RNA. It is involved in the processing of ribosomal RNA precursors and of some mRNAs. RNase III is evolutionary related [2] to the following proteins: - Fission yeast pac1, a ribonuclease that probably inhibits mating and meiosis by degrading a specific mRNA required for sexual development. - Yeast ribonuclease III (gene RNT1), a dsRNA-specific nuclease that cleaves eukaryotic preribosomal RNA at various sites. - Caenorhabditis elegans hypothetical protein F26E4.13. - Paramecium bursaria chlorella virus 1 protein A464R. - Synechocystis strain PCC 6803 hypothetical protein slr0346. - Fission yeast hypothetical protein SpAC8A4.08c, a protein with a N-terminal helicase domain and a C-terminal RNase III domain. - Caenorhabditis elegans hypothetical protein K12H4.8, a protein with the same structure as SpAC8A4.08c.These proteins share regions of sequence similarity; one of which is a highly conserved stretch of 9 residues which has been developed as a signature pattern.

Consensus pattern: [DEQ]-[RQ]-[LM]-E-[FYW]-[LV]-G-D-[SAR]-

[1] Nashimoto H., Uchida H. Mol. Gen. Genet. 201:25-29(1985).
[2] Mian I.S. Nucleic Acids Res. 25:3187-3195(1997).

490. Rieske iron-sulfur protein signatures

Ubiquinol-cytochrome c reductase (EC 1.10.2.2) (also known as the bc1 complexor complex III) is one of the electron transport chains of mitochondria and of some aerobic prokaryotes; it catalyzes the oxidoreduction of ubiquinol and cytochrome c. In the chloroplast of plants and in cyanobacteria plastoquinone-plastocyanin reductase (EC 1.10.99.1) (also known as the b6f complex) is functionally similar and catalyzes the oxidoreduction of plastoquinol and cytochrome f. One of the components of these electron transfer systems is an iron-sulfur protein with a 2Fe-2S cluster, which is called the Rieske protein [1,2]. The Rieske protein contains approximately 190 amino acid residues. The iron-sulfur cluster is complexed to the protein through cysteine and histidine residues. Two perfectly conserved regions in Rieske proteins contains all the residuesthat bind the iron-sulfur cluster. Both regions contain two cysteines and a histidine. The first cysteine and the histidine are 2Fe-2S ligands while the remaining cysteines form a disulfide bond [3]. Two conserved regions were selected as signature patterns.

Consensus pattern: C-[TK]-H-L-G-C-[LIVST] [The first C and the H are 2Fe-2S ligands] [The second C is involved in a disulfide bond]
Consensus pattern: C-P-C-H-x-[GSA] [The first C and the H are 2Fe-2S ligands] [The second C is involved in a disulfide bond]

[1] Gatti F.L., Meinhardt S.W., Ohnishi T., Tzagoloff A. J. Mol. Biol. 205:421-435(1989).
[2] Kallas T., Spiller S., Malkin R. Proc. Natl. Acad. Sci. U.S.A. 85:5794-5798(1988).
[3] Iwata S., Saynovits M., Link T.A., Michel H. Structure 4:567-579(1996).

491. Ribosomal protein L1 signature

Ribosomal protein L1 is the largest protein from the large ribosomal subunit.In Escherichia coli, L1 is known to bind to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1, 2], groups: - Eubacterial L1. - Algal and plant chloroplast L1. - Cyanelle L1. - Archaebacterial L1. - Vertebrate L10A. - Yeast SSM1.As a signature pattern, the best conserved region was selected located in the central section of these proteins. It is located at the end of an alpha helix thought to be involved in RNA-binding.

Consensus pattern: [IM]-x(2)-[LIVA]-x(2,3)-[LIVM]-G-x(2)-[LMS]-[GSNH]-[PTKR]-[KRAV]-G-x-[LIMF]-P-[DENSTKQ]

[1] Nikonov S.V., Nevskaya N., Eliseikina I.A., Fomenkova N.P., Nikulin A., Ossina N., Garber M., Jonsson B.-H., Briand C., Al-Karadaghi S., Svensson L.A., Aevarsson A., Liljas A. EMBO J. 15:1350-1359(1996).

[2] Olvera J., Wool I.G. 2.3.CO;2-"Biochem. Biophys. Res. Commun. 220:954-957(1996).

09555126.061802

492. Ribosomal protein L10 signature

Ribosomal protein L10 is one of the proteins from the large ribosomal subunit. L10 is a protein of 162 to 185 amino-acid residues which has only been found so far in eubacteria. A conserved region located in the N-terminal section of these proteins was used as a signature pattern.

Consensus pattern: [DEH]-x(2)-[GS]-[LIVMF]-[STN]-[VA]-x-[DEQK]-[LIVMA]-x(2)-[LIM]-R

493. Ribosomal protein L10e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Vertebrate L10 (QM) [1]. - Plant L10. - Caenorhabditis elegans L10 (F10B5.1). - Yeast L10 (QSR1). - Methanococcus jannaschii MJ0543.These proteins have 174 to 232 amino-acid residues. A conserved region located in the central section was selected as a signature pattern.

Consensus pattern: R-x-A-[FYW]-G-K-[PA]-x-G-x(2)-A-R-V

[1] Chan Y.-L., Diaz J.-J., Denoroy L., Madjar J.-J., Wool I.G. <u>2.3.CO;2-"Biochem. Biophys. Res. Commun. 255:952-956(1996).</u>

494. Ribosomal protein L11 signature

Ribosomal protein L11 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L11 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups:

- Eubacterial L11.
- Plant chloroplast L11 (nuclear-encoded).
- Read algal chloroplast L11.
- Cyanelle L11.
- Archaebacterial L11.
- Mammalian L12.
- Plants L12.
- Yeast L12 (YL15).

L11 is a protein of 140 to 165 amino-acid residues. A conserved region located in the C-terminal section of these proteins was selected as a signature pattern. In Escherichia coli, the C-terminal half of L11 has been shown [3] to be in an extended and loosely folded conformation and is likely to be buried within the ribosomal structure.

Consensus pattern: [RKN]-x-[LIVM]-x-G-[ST]-x(2)-[SNQ]-[LIVM]-G-x(2)-[LIVM]-x(0,1)-[DENG]

[1] Pucciarelli G., Remacha M., Ballesta J.P.G.; Nucleic Acids Res. 18:4409-4416(1990).
[2] Otaka E., Hashimoto T., Mizuta K., Suzuki K.; Protein Seq. Data Anal. 5:301-313(1993).
[3] Choli T. Biochem. Int. 19:1323-1338(1989).

09555326.062600

495. Ribosomal protein L7/L12 C-terminal domain

[1] Leijonmarck M, Liljas A; J Mol Biol 1987;195:555-579.

496. Ribosomal protein L13 signature

Ribosomal protein L13 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L13 is known to be one of the early assembly proteins of the 50S ribosomal subunit. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L13.

- Plant chloroplast L13 (nuclear-encoded). - Red algal chloroplast L13.
- Archaebacterial L13. - Mammalian L13a (Tum P198). - Yeast Rp22 and Rp23.

L11 is a protein of 140 to 250 amino-acid residues. As a signature pattern, a conserved region was selected located in the C-terminal section of these proteins.

Consensus pattern: [LIVM]-[KRV]-[GK]-M-[LIV]-[PS]-x(4,5)-[GS]-[NQEKRA]-x(5)-[LIVM]-x-[AIV]-[LFY]-x-[GDN]

[1] Chan Y.-L., Olvera J., Glueck A., Wool I.G. J. Biol. Chem. 269:5589-5594(1994).

497. Ribosomal protein L13e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:

- Vertebrate L13 (was previously known as Breast Basic Conserved protein 1 (BBC1)). - Drosophila L13. - Plant L13. - Yeast probable L13 (YM9375.11c).

These proteins have 199 to 218 amino-acid residues. As a signature pattern, a stretch of about 16 residues in the first third of these proteins selected.

-Consensus pattern: [KR]-Y-x(2)-K-[LIVM]-R-[STA]-G-[KR]-G-F-[ST]-L-x-E

[1] Olvera J., Wool I.G. Biochem. Biophys. Res. Commun. 201:102-107(1994).

498. Ribosomal protein L14 signature

Ribosomal protein L14 is one of the proteins from the large ribosomal subunit. In eubacteria, L14 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L14. - Algal and plant chloroplast L14. - Cyanelle L14. - Archaebacterial L14. - Yeast L17A. - Mammalian L23. - Caenorhabditis elegans L23 (B0336.10). - Higher eukaryotes mitochondrial L14. - Yeast mitochondrial Yml38 (gene MRPL38).

L14 is a protein of 119 to 137 amino-acid residues. As a signature pattern, a conserved region located in the C-terminal half of these proteins was selected.

-Consensus pattern: [GA]-[LIV](3)-x(9,10)-[DNS]-G-x(4)-[FY]-x(2)-[NT]-x(2)-V-[LIV]

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

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499. Ribosomal protein L15 signature

Ribosomal protein L15 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L15 is known to bind the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L15. - Plant chloroplast L15 (nuclear-encoded). - Archaebacterial L15. - Vertebrate L27a. - Tetrahymena thermophila L29. - Fungi L27a (L29, CRP-1, CYH2).

L15 is a protein of 144 to 154 amino-acid residues. As a signature pattern, a conserved region was selected in the C-terminal section of these proteins.

-Consensus pattern: K-[LIVM](2)-[GASL]-x-[GT]-x-[LIVMA]-x(2,5)-[LIVM]-x-[LIVMF]-x(3,4)-[LIVMFCA]-[ST]-x(2)-A-x(3)-[LIVM]-x(3)-G

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

500. Ribosomal protein L15e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:

- Mammalian L15. - Insect L15. - Plant L15. - Yeast YL10 (L13) (Rp15r).
- Thermoplasma acidophilum L15.

These proteins have about 200 amino acid residues. As a signature pattern, a conserved region was selected located in the central section.

-Consensus pattern: [DE]-[KR]-A-R-x-L-G-[FY]-x-[SAP]-x(2)-G-[LIVMFY](4)-R-x-R-[IV]-x-R-G

[1] Zwickl P., Lupas A., Baumeister W. Biochem. Biophys. Res. Commun. 209:684-688(1995).

501. Ribosomal protein L17 signature

Ribosomal protein L17 is one of the proteins from the large ribosomal subunit. L17 belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L17.

- Yeast mitochondrial YmL8 (gene MRPL8).

Eubacterial L17 is a protein of 120 to 130 amino-acid residues. Yeast YmL8 is twice larger (238 residues), the sequence of its N-terminal half is colinear with that of eubacterial L17. As a signature pattern, a conserved region in the N-terminal section was selected.

-Consensus pattern: I-x-[ST]-[GT]-x(2)-[KR]-x-K-x(6)-[DE]-x-[LIMV]-[LIVMT]-T-x-[STAG]-[KR]

502. Ribosomal protein L18e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate L18 (known as L14 in Xenopus) [1]. - Plant L18.
- Yeast L18 (Rp28). - Halobacterium marismortui Hl29.
- Sulfolobus acidocaldarius Hl29e.

These proteins have 115 to 187 amino-acid residues., A stretch of about 13 residues in the first third of these proteins has been selected as a signature pattern.

-Consensus pattern: [KRE]-x-L-x(2)-[PS]-[KR]-x(2)-[RH]-[PSA]-x-[LIVM]-[NS]-[LIVM]-x-[RK]-[LIVM]

[1] Puder M., Barnard G.F., Staniunas R.J., Steele G.D. Jr., Chen L.B. Biochim. Biophys. Acta 1216:134-136(1993).

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503. Ribosomal L18p family

It has been shown that the amino terminal 93 amino acids of Swiss:P09895 are necessary and sufficient to bind 5S rRNA in vitro. The carboxyl-terminal half of the protein, comprising amino acids 151-296, serves to localize the protein to the nucleolus [1].

Number of members: 26

[1]

Medline: 96212235

Distinct domains in ribosomal protein L5 mediate 5 S rRNA binding and nucleolar localization.

Michael WM, Dreyfuss G;

J Biol Chem 1996;271:11571-11574.

504. Ribosomal protein L19 signature

Ribosomal protein L19 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L19 is known to be located at the 30S-50S ribosomal subunit interface and may play a role in the structure and function of the aminoacyl-tRNA binding site. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L19.

- Red algal chloroplast L19. - Cyanelle L19.

L19 is a protein of 120 to 130 amino-acid residues.,

A conserved region in the C-terminal section has been selected as a signature pattern.

-Consensus pattern: [LIVM]-x-[KRGTI]-x-[GSAI]-[KRQDA]-[VG]-[RSN]-X(0,1)-[KR]-[SA]-[KY]-[KLI]-[LYS]-Y-[LIM]-R

505. Ribosomal protein L19e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian ribosomal protein L19 [1]. - Drosophila ribosomal protein L19 [2].
- Slime mold (D. discoideum) vegetative specific protein V14 [3].
- Yeast ribosomal protein L19 (YL14). - Archebacterial ribosomal protein L19E.

These proteins have 148 to 203 amino-acid residues.

A stretch of about 20 residues in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: Q-[KR]-R-[LIVM]-x-[SA]-x(4)-[CV]-G-x(3)-[IV]-[WK]-[LIVF]-[DN]-P

[1] Chan Y.-L., Lin A., McNally J., Peleg D., Meyuhas O., Wool I.G. J. Biol. Chem. 262:1111-1115(1987).[2] Hart K., Klein T., Wilcox M. Mech. Dev. 43:101-110(1993).[3] Singleton C.K., Manning S.S., Ken R. Nucleic Acids Res. 17:9679-9692(1989).

506. Ribosomal protein L1e signature (Ribosomal_L4)

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists [1,2,3, 4] of: - Vertebrate L1 (L4). - Drosophila L1. - Plant L1. - Yeast L2 (Rp2).
- Fission yeast L2. - Halobacterium marismortui HmaL4 (HL6).
- Methanococcus jannaschii MJ0177.

These proteins have 246 (archaebacteria) to 427 (human) amino acids. A conserved region in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: N-x(3)-[KRM]-x(2)-A-[LIVT]-x-S-A-[LIV]-x-A-[ST]-[SGA]-x(7)-[RK]-[GS]-H

[1] Rafti F., Gargiulo G., Manzi A., Malva C., Graziani F. Nucleic Acids Res. 17:456-456(1989).[2] Presutti C., Villa T., Bozzoni I. Nucleic Acids Res. 21:3900-3900(1993).

[3] Bagni C., Mariottini P., Annesi F., Amaldi F. Biochim. Biophys. Acta 1216:475-478(1993).

[3] Arndt E., Kroemer W., Hatakeyama T. J. Biol. Chem. 265:3034-3039(1990).

507. Ribosomal protein L2 signature

Ribosomal protein L2 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L2 is known to bind to the 23S rRNA and to have peptidyltransferase activity. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial L2.
- Algal and plant chloroplast L2. - Cyanelle L2. - Archaebacterial L2.
- Plant L2. - Slime mold L2. - Marchantia polymorpha mitochondrial L2.
- Paramecium tetraurelia mitochondrial L2. - Fission yeast K5, K37 and KD4.
- Yeast YL6. - Vertebrate L8.

The best conserved region located in the C-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: P-x(2)-R-G-[STAIV](2)-x-N-[APK]-x-[DE]

[1] Marty I., Meyer Y. Nucleic Acids Res. 20:1517-1522(1992).

[2] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

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508. Ribosomal protein L20 signature

Ribosomal protein L20 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L20 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L20. - Algal and plant chloroplast L20.
- Cyanelle L20.

L20 is a protein of about 120 amino-acid residues. A conserved region located in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: K-x(3)-[KRC]-x-[LIVM]-W-[IV]-[STNALV]-R-[LIVM]-[NS]-x(3)-[RKHS]

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K.
Protein Seq. Data Anal. 5:301-313(1993).

509. Ribosomal protein L21e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L21 [1]. - Entamoeba histolytica L21 [2].
- Caenorhabditis elegans L21 (C14B9.7). - Yeast L21E (URP1) [3].
- Halobacterium marismortui HL31 [4].

These proteins have 160 (eukaryotes) or 95 (archebacteria) amino-acid residues. A conserved region in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: G-[DE]-x-V-x(10)-[GV]-x(2)-[FYH]-x(2)-[FY]-x-G-x-T-G

[1] Devi K.R.G., Chan Y.-L., Wool I.G.
Biochem. Biophys. Res. Commun. 162:364-370(1989).

[2] Petter R., Rozenblatt S., Nuchamowitz Y., Mirelman D.
Mol. Biochem. Parasitol. 56:329-333(1992).

[3] Jank B., Waldherr M., Schweyen R.J. Curr. Genet. 23:15-18(1993).

[4] Hatakeyama T., Kimura M. Eur. J. Biochem. 172:703-711(1988).

510. Ribosomal protein L21 signature

Ribosomal protein L21 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L21 is known to bind to the 23S rRNA in the presence of L20. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L21.

- Marchantia polymorpha chloroplast L21. - Cyanelle L21.
- Spinach chloroplast L21 (nuclear-encoded).

Eubacterial L21 is a protein of about 100 amino-acid residues, the mature form of the spinach chloroplast L21 has 200 residues. A conserved region located in the C-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [IVT]-x(3)-[KR]-x(3)-[KRQ]-K-x(6)-G-[HF]-R-[RQ]-x(2)-[ST]

511. Ribosomal protein L22 signature

Ribosomal protein L22 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L22 is known to bind 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial L22.

- Algal and plant chloroplast L22 (in legumes L22 is encoded in the nucleus instead of the chloroplast). - Cyanelle L22. - Archaebacterial L22.
- Mammalian L17. - Plant L17. - Yeast YL17.

A conserved region located in the C- terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [RKQN]-x(4)-[RH]-[GAS]-x-G-[KRQS]-x(9)-[HDN]-[LIVM]-x-[LIVMS]-x-[LIVM]

[1] Gantt J.S., Baldauf S.L., Calie P.J., Weeden N.F., Palmer J.D. EMBO J. 10:3073-3078(1991).[2] Madsen L.H., Kreiberg J.D., Gausing K. Curr. Genet. 19:417-422(1991).

[3] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

512. Ribosomal protein L23 signature

Ribosomal protein L23 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L23 is known to bind a specific region on the 23S rRNA; in yeast, the corresponding protein binds to a homologous site on the 26S rRNA [1]. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [2,3,4], groups: - Eubacterial L23.

- Algal and plant chloroplast L23. - Archaebacterial L23. - Mammalian L23A.
- Caenorhabditis elegans L23A (F55D10.2). - Fungi L25.

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- Yeast mitochondrial YmL41 (gene MRPL41 or MRP20).

A small conserved region in the C-terminal section of these proteins, which is probably involved in rRNA-binding has been selected as a signature pattern [2].

-Consensus pattern: [RK](2)-[AM]-[IVFYT]-[IV]-[RKT]-L-[STANEQK]-x(7)-[LIVMFT]

[1] El Baradi T.T.A.L., Raue H.A., van de Regt C.H.F., Verbree E.C., Planta R.J. EMBO J. 4:210-2107(1985).

[2] Raue H.A., Otaka E., Suzuki K. J. Mol. Evol. 28:418-426(1989).

[3] Fearon K., Mason T.L. J. Biol. Chem. 267:5162-5170(1992).

[4] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

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513. Ribosomal protein L24 signature

Ribosomal protein L24 is one of the proteins from the large ribosomal subunit. L24 belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L24.

- Plant chloroplast L24 (nuclear-encoded). - Red algal L24. - Vertebrate L26.
- Yeast L26 (YL33). - Archaebacterial HmaL24 (HL15).
- A probable ribosomal protein from Sulfolobus acidocaldarius [1].

In their mature form, these proteins have 103 to 150 amino-acid residues. A conserved stretch of 20 residues in their N-terminal section has been selected as a signature pattern.

-Consensus pattern: [GDEN]-D-x-V-x-[IV]-[LIVMA]-x-G-x(2)-[KRA]-[GNQ]-x(2,3)-[GA]-x-[IV]

[1] Ouzounis C., Kyrpides N., Sander C. Nucleic Acids Res. 23:565-570(1995).

514. Ribosomal protein L24e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists [1] of:

- Mammalian ribosomal protein L24.

- Yeast ribosomal protein L30A/B (Rp29) (YL21).
- Kluyveromyces lactis ribosomal protein L30.
- Arabidopsis thaliana ribosomal protein L24 homolog.
- Haloarcula marismortui ribosomal protein HL21/HL22.
- Methanococcus jannaschii MJ1201.

These proteins have 60 to 160 amino-acid residues. The most conserved region, which is located in the N-terminal region of these proteins has been selected as a signature pattern.

-Consensus pattern: [FY]-x-[GSH]-x(2)-[IV]-x-P-G-x-G-x(2)-[FYV]-x-[KRHE]-x-D

[1] Chan Y.-L., Olvera J., Wool I.G.
Biochem. Biophys. Res. Commun. 202:1176-1180(1994).

515. Ribosomal protein L27 signature

Ribosomal protein L27 is one of the proteins from the large ribosomal subunit. L27 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial L27.

- Plant chloroplast L27 (nuclear-encoded). - Algal chloroplast L27.
- Yeast mitochondrial YmL2 (gene MRPL2 or MRP7).

The schematic relationship between these groups of proteins is shown below.

Eub. L27 NxxxxxxxxxAlgal L27 Nxxxxxxxxx
Plant L27 tttttNxxxxxxxxxxxxxx
Yeast MRP7 tttNxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
***'t': transit peptide.
'N': N-terminal of mature protein.'*': position of the pattern.

-Consensus pattern: G-x-[LIVM](2)-x-R-Q-R-G-x(5)-G

[1] Elhag G.A., Bourque D.P. Biochemistry 31:6856-6864(1992).
[2] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).

516. Ribosomal L28 family

The ribosomal 28 family includes L28 proteins from bacteria and chloroplasts. The L24 protein from yeast Swiss:P36525 also contains a region of similarity to prokaryotic L28 proteins. L24 from yeast is also found in the large ribosomal subunit

Number of members: 24

517. Ribosomal protein L29 signature

Ribosomal protein L29 is one of the proteins from the large ribosomal subunit. L29 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L29. - Red algal L29.

- Archaebacterial L29. - Mammalian L35 - Caenorhabditis elegans L35 (ZK652.4).
- Yeast L35.

L29 is a protein of 63 to 138 amino-acid residues.

A conserved region located in the central section of L29 has been selected as a signature pattern.

-Consensus pattern: [KNQS]-[PSTL]-x(2)-[LIMFA]-[KRGSAN]-x-[LIVYSTA]-[KR]-[KRHQS]-[DESTANRL]-[LIV]-A-[KRCQVT]-[LIVMA]

[1] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).

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518. Ribosomal protein L3 signature

Ribosomal protein L3 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L3 is known to bind to the 23S rRNA and may participate in the formation of the peptidyltransferase center of the ribosome. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3,4], groups: - Eubacterial L3. - Red algal L3. - Cyanelle L3.

- Archaebacterial Halobacterium marismortui HmaL3 (HL1).
- Yeast L3 (also known as trichodermin resistance protein) (gene TCM1).
- Arabidopsis thaliana L3 (genes ARP1 and ARP2). - Mammalian L3 (L4).
- Mammalian mitochondrial L3. - Yeast mitochondrial YmL9 (gene MRPL9).

A conserved region located in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: [FL]-x(6)-[DN]-x(2)-[AGS]-x-[ST]-x-G-[KRH]-G-x(2)-G-x(3)-R

[1] Arndt E., Kroemer W., Hatakeyama T. J. Biol. Chem. 265:3034-3039(1990).

[2] Graack H.-R., Grohmann L., Kitakawa M., Schaefer K.L., Kruft V.
Eur. J. Biochem. 206:373-380(1992).

[3] Herwig S., Kruft V., Wittmann-Liebold B.
Eur. J. Biochem. 207:877-885(1992).

[4] Otaka E., Hashimoto T., Mizuta K., Suzuki K.
Protein Seq. Data Anal. 5:301-313(1993).

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519. Ribosomal protein L30 signature

Ribosomal protein L30 is one of the proteins from the large ribosomal subunit. L30 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L30. - Archaebacterial L30.
- Drosophila L7. - Slime mold L7. - Mammalian L7. - Fungi L7 (YL8).
- Yeast mitochondrial L33.

L30 from eubacteria are small proteins of about 60 residues, those from archaebacteria are proteins of about 150 residues. Eukaryotic L7 are proteins of about 250 to 270 residues. The schematic relationship between the three groups of proteins is shown below.Eub. L30 NxxxxxxxxxxC

Arc. L30 NxxxxxxxxxxxxxxxxxxxxxxxxxxxxC

Euk. L7 NxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxC

*******'*': position of the pattern.

The signature pattern for this family of ribosomal proteins spans the N-terminal half of the region common to all these proteins.

-Consensus pattern: [IVT]-[LIVM]-x(2)-[LF]-x-[LI]-x-[KRHQEG]-x(2)-[STNQH]-x-[IVT]-x(10)-[LMS]-[LIV]-x(2)-[LIVA]-x(2)-[LMFY]-[IVT]

[1] Mizuta K., Hashimoto T., Otaka E.
Nucleic Acids Res. 20:1011-1016(1992).

520. Ribosomal protein L31 signature

Ribosomal protein L31 is one of the proteins from the large ribosomal subunit.

L31 is a protein of 66 to 97 amino-acid residues which has only been found so far in eubacteria and in some algal chloroplasts.

A conserved region located in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: H-P-F-[FY]-[TI]-x(9)-G-R-[AIV]-x-[KRQ]

521. Ribosomal protein L31e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L31 [1]. - Chlamydomonas reinhardtii L31. - Yeast L34.
- Halobacterium marismortui HL30 [2].

These proteins have 87 to 128 amino-acid residues.

A conserved region, located in the central section has been selected as a signature pattern.

-Consensus pattern: V-[KR]-[LIVM]-x(3)-[LIVM]-N-x-[AKH]-x-W-x-[KR]-G

[1] Tanaka T., Kuwano Y., Kuzumaki T., Ishikawa K., Ogata K. Eur. J. Biochem. 162:45-48(1987).[2] Bergmann U., Arndt E. Biochim. Biophys. Acta 1050:56-60(1990).

522. Ribosomal protein L33 signature

Ribosomal protein L33 is one of the proteins from the large ribosomal subunit.

In Escherichia coli, L33 has been shown to be on the surface of 50S subunit.

L33 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial L33.

- Algal and plant chloroplast L33. - Cyanelle L33.

L33 is a small protein of 49 to 66 amino-acid residues. A conserved region located in the central section of L33 has been selected as a signature pattern.

-Consensus pattern: Y-x-[ST]-x-[KR]-[NS]-x(4)-[PATQ]-x(1,2)-[LIVM]-[EA]-x(2)-K-[FY]-[CSD]

[1] Kruft V., Kapp U., Wittmann-Liebold B. Biochimie 73:855-860(1991).

[2] Sharp P.M. Gene 139:129-130(1994).

[3] Otaka E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

523. Ribosomal protein L34 signature

Ribosomal protein L34 is one of the proteins from the large subunit of the prokaryotic ribosome. It is a small basic protein of 44 to 51 amino-acid residues [1]. L34 belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups: - Eubacterial L34.

- Red algal chloroplast L34. - Cyanelle L34.

A conserved region that corresponds to the N-terminal half of L34 has been selected as a signature pattern.

-Consensus pattern: K-[RG]-T-[FYWL]-[EQS]-x(5)-[KRHS]-x(4,5)-G-F-x(2)-R

[1] Old I.G., Margarita D., Saint Girons I.
Nucleic Acids Res. 20:6097-6097(1992).

524. Ribosomal protein L34e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L34. - Mosquito L31 [1]. - Plant L34 [2].
- Yeast putative ribosomal protein YIL052c. - Methanococcus jannaschii MJ0655.

These proteins have 89 to 129 amino-acid residues.

A conserved region located in the N-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: Y-x-[ST]-x-S-[NY]-x(5)-[KR]-T-P-G

[1] Lan Q., Niu L.L., Fallon A.M.
Biochim. Biophys. Acta 1218:460-462(1994).

[2] Gao J., Kim S.R., Chung Y.Y., Lee J.M., An G.
Plant Mol. Biol. 25:761-770(1994).

525. Ribosomal protein L35Ae signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate L35A. - Caenorhabditis elegans L35A (F10E7.7).
- Yeast L37A/L37B (Rp47). - Pyrococcus woesei L35A homolog [1].

These proteins have 87 to 110 amino-acid residues.

A highly conserved stretch of 22 residues in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: G-K-[LIVM]-x-R-x-H-G-x(2)-G-x-V-x-A-x-F-x(3)-[LI]-P

[1] Ouzounis C., Kyrpides N., Sander C.
Nucleic Acids Res. 23:565-570(1995).

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526. Ribosomal protein L36 signature

Ribosomal protein L36 is the smallest protein from the large subunit of the prokaryotic ribosome. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial L36. - Algal and plant chloroplast L36. - Cyanelle L36.L36 is a small basic and cysteine-rich protein of 37 amino-acid residues. As a signature pattern, a conserved region that corresponds to positions 11 to 36 in L36 and includes three conserved cysteine residues has been developed.

Consensus pattern: C-x(2)-C-x(2)-[LIVM]-x-R-x(3)-[LIVMN]-x-[LIVM]-x-C-x(3,4)- [KR]-H-x-Q-x-Q-

[1] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

527. Ribosomal protein L36e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Mammalian L36 [1].
- Drosophila L36 (M(1)1B). - Caenorhabditis elegans L36 (F37C12.4).
- Candida albicans L39. - Yeast YL39.

These proteins have 99 to 104 amino acids.

A conserved region in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: P-Y-E-[KR]-R-x-[LIVM]-[DE]-[LIVM](2)-[KR]

[1] Chan Y.-L., Paz V., Olvera J., Wool I.G.
Biochem. Biophys. Res. Commun. 192:849-853(1993).

528. Ribosomal protein L39e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L39 [1]. - Plants L39. - Yeast L46 [2]. - Archebacterial L39e [3].

These proteins are very basic. About 50 residues long, they are the smallest proteins of eukaryotic-type ribosomes. A conserved region in the C-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [KRA]-T-x(3)-[LIVM]-[KRQF]-x-[NHS]-x(3)-R-[NHY]-W-R-R

[1] Lin A., McNally J., Wool I.G. J. Biol. Chem. 259:487-490(1984).

[2] Leer R.J., van Raamsdonk-Duin M.M.C., Kraakman P., Mager W.H., Planta R.J. Nucleic Acids Res. 13:701-709(1985).

[3] Ramirez C., Louie K.A., Matheson A.T. FEBS Lett. 250:416-418(1989).

529. Ribosomal L40e family

Bovine L40 has been identified as a secondary RNA binding protein [1]. L40 is fused to a ubiquitin protein [2].

Number of members: 27

[1]

Medline: 88203200

RNA binding proteins of the large subunit of bovine mitochondrial ribosomes.

Piatyszek MA, Denslow ND, O'Brien TW;

Nucleic Acids Res 1988;16:2565-2583.

[2]Medline: 96011832

The carboxyl extensions of two rat ubiquitin fusion proteins are ribosomal proteins S27a and L40.

Chan YL, Suzuki K, Wool IG;

Biochem Biophys Res Commun 1995;215:682-690.

530. (Ribosomal L44) Ribosomal protein L44e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L44 [1]. - Trypanosoma brucei L44.
- Caenorhabditis elegans L44 (C09H10.2). - Fungal L44 (L41).
- Halobacterium marismortui LA [2].

These proteins have 92 to 105 amino-acid residues.

A conserved region located in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: K-x-[TV]-K-K-x(2)-L-[KR]-x(2)-C

[1] Gallagher M.J., Chan Y.-L., Lin A., Wool I.G. DNA 7:269-273(1988).

[2] Bergmann U., Wittmann-Liebold B.
Biochim. Biophys. Acta 1173:195-200(1993

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531. Ribosomal protein L5 signature

Ribosomal protein L5 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L5 is known to be involved in binding 5S RNA to the large ribosomal subunit. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3,4], groups: - Eubacterial L5.

- Algal chloroplast L5. - Cyanelle L5. - Archaebacterial L5. - Mammalian L11.
- Tetrahymena thermophila L21. - Slime mold L5 (V18). - Yeast L16 (39A).
- Plants mitochondrial L5.

L5 is a protein of about 180 amino-acid residues.

A conserved region, located in the first third of these proteins has been selected as a signature pattern.

-Consensus pattern: [LIVM]-x(2)-[LIVM]-[STAVC]-[GE]-[QV]-x(2)-[LIVMA]-x-[STC]-x-[STAG]-[KRH]-x-[STA]

[1] Hatakeyama T., Hatakeyama T. Biochim. Biophys. Acta 1039:343-347(1990).

[2] Rosendahl G., Andreasen P.H., Kristiansen K. Gene 98:161-167(1991).

[3] Yang D., Gunther I., Matheson A.T., Auer J., Spicker G., Boeck A.
Biochimie 73:679-682(1991).

[4] Otaka E., Hashimoto T., Mizuta K., Suzuki K.

Protein Seq. Data Anal. 5:301-313(1993).

532. ribosomal L5P family C-terminus

This region is found associated with Ribosomal_L5.

Number of members: 60

533. Ribosomal protein L6 signatures

Ribosomal protein L6 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L6 is known to bind directly to the 23S rRNA and is located at the aminoacyl-tRNA binding site of the peptidyltransferase center. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3,4], groups: - Eubacterial L6.

- Algal chloroplast L6.
- Cyanelle L6.
- Archaebacterial L6.
- Marchantia polymorpha mitochondrial L6.
- Yeast mitochondrial YmL6 (gene MRPL6).
- Mammalian L9.
- Drosophila L9.
- Plants L9.
- Yeast L9 (YL11).

While all the above proteins are evolutionary related it is very difficult to derive a pattern that will find them all. Two patterns were therefore created, the first to detect eubacterial, cyanelle and mitochondrial L6, the second to detect archaebacterial L6 as well as eukaryotic L9.

-Consensus pattern: [PS]-[DENS]-x-Y-K-[GA]-K-G-[LIVM]

-Consensus pattern: Q-x(3)-[LIVM]-x(2)-[KR]-x(2)-R-x-F-x-D-G-[LIVM]-Y-[LIVM]-x(2)-[KR]

[1] Suzuki K., Olvera J., Wool I.G. Gene 93:297-300(1990).

[2] Schwank S., Harrer R., Schueller H.-J., Schweizer E. Curr. Genet. 24:136-140(1993).

[3] Golden B.L., Ramakrishnan V., White S.W. EMBO J. 12:4901-4908(1993).

[4] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

534. Ribosomal protein L6e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian ribosomal protein L6 (L6 was previously known as TAX-responsive enhancer element binding protein 107).
- Caenorhabditis elegans ribosomal protein L6 (R151.3).
- Yeast ribosomal protein YL16A/YL16B.
- Mesembryanthemum crystallinum ribosomal protein YL16-like.

These proteins have 175 (yeast) to 287 (mammalian) amino acids. A highly conserved region in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: N-x(2)-P-L-R-R-x(4)-[FY]-V-I-A-T-S-x-K

535. Ribosomal protein L7Ae signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate L7A (SURF3) [1]. - Plant L7A. - Yeast L7A (YL5) (Rp6).
- Yeast protein NHP2 [2]. - Yeast hypothetical protein YEL026w.
- Bacillus subtilis hypothetical protein ylxQ. - Halobacterium marismortui Hs6.
- Methanococcus jannaschii MJ1203.

These proteins have 100 to 265 amino-acid residues.

A conserved region located in the central section has been selected as a signature pattern.

-Consensus pattern: [CA]-x(4)-[IV]-P-[FY]-x(2)-[LIVM]-x-[GSQ]-[KRQ]-x(2)-L-G

[1] Colombo P., Yon J., Garson K., Fried M.
Proc. Natl. Acad. Sci. U.S.A. 89:6358-6362(1992).

[2] Kolodrubetz D., Burgum A. Yeast 7:79-90(1991).

536. Ribosomal protein L9 signature

Ribosomal protein L9 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L9 is known to bind directly to the 23S rRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial L9. - Cyanobacterial L9.

- Plant chloroplast L9 (nuclear-encoded). - Red algal chloroplast L9.

A conserved region, located in the N-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: G-x(2)-[GN]-x(4)-V-x(2)-G-[FY]-x(2)-N-[FY]-L-x(5)-[GA]-x(3)-[STN]

[1] Hoffman D.W., Davies C., Gerchman S.E., Kycia J.H., Porter S.J., White S.W., Ramakrishnan V. EMBO J. 13:205-212(1994).

[2] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

537. Ribosomal protein S10 signature

Ribosomal protein S10 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S10 is known to be involved in binding tRNA to the ribosomes. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial S10.

- Algal chloroplast S10. - Cyanelle S10. - Archaebacterial S10.
- Marchantia polymorpha and Prototheca wickerhamii mitochondrial S10.
- Arabidopsis thaliana mitochondrial S10 (nuclear encoded). - Vertebrate S20.
- Plant S20. - Yeast URP2.

S10 is a protein of about 100 amino-acid residues.

A conserved region located in the center of these proteins has been selected as a signature pattern.

-Consensus pattern: [AV]-x(3)-[GDNSR]-[LIVMSTA]-x(3)-G-P-[LIVM]-x-[LIVM]-P-T

[1] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

538. Ribosomal protein S11 signature

Ribosomal protein S11 [1] plays an essential role in selecting the correct tRNA in protein biosynthesis. It is located on the large lobe of the small ribosomal subunit. S11 belongs to a family of ribosomal proteins which, on the basis of sequence similarities, groups [2]: - Eubacterial S11.

- Algal and plant chloroplast S11. - Cyanelle S11. - Archaebacterial S11.
- Marchantia polymorpha and Prototheca wickerhamii mitochondrial S11.
- Acanthamoeba castellanii mitochondrial S11. - Neurospora crassa S14 (crp-2).
- Yeast S14 (RP59 or CRY1).
- Mammalian, Drosophila, Trypanosoma, and plant S14.
- Caenorhabditis elegans S14 (F37C12.9).

One of the best conserved regions in these proteins was selected as a signature pattern.

-Consensus pattern: [LIVMF]-x-[GSTAC]-[LIVMF]-x(2)-[GSTAL]-x(0,1)-[GSN]-[LIVMF]-x-[LIVM]-x(4)-[DEN]-x-T-P-x-[PA]-[STCH]-[DN]

[1] Kimura M., Kimura J., Hatakeyama T. FEBS Lett. 240:15-20(1988).

[2] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).

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539. Ribosomal protein S12 signature

Ribosomal protein S12 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S12 is known to be involved in the translation initiation step. It is a very basic protein of 120 to 150 amino-acid residues. S12 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial S12. - Archaebacterial S12.

- Algal and plant chloroplast S12. - Cyanelle S12.
- Protozoa and plant mitochondrial S12. - Yeast S28.
- Drosophila mitochondrial protein tko (Technical KnockOut). - Mammalian S23.

The best conserved regions in these proteins, located in the center of each sequence have been selected as a signature pattern.

-Consensus pattern: [RK]-x-P-N-S-[AR]-x-R

[1] Otaka E., Hashimoto T., Mizuta K.

Protein Seq. Data Anal. 5:285-300(1993).

540. Ribosomal protein S12e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Vertebrate S12 [1].
 - Trypanosoma brucei S12 [2]. - Caenorhabditis elegans S12 (F54E7.2).
 - Drosophila S12. - Yeast S12.

These proteins have 130 to 150 amino acids.

A conserved region in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: A-L-[KRQP]-x-V-L-x(2)-[SA]-x(3)-[DN]-G-L

[1] Lin A., Chan Y.-L., Jones R., Wool I.G.
 J. Biol. Chem. 262:14343-14351(1987).[2] Marchal C., Ismaili N., Pays E.
 Mol. Biochem. Parasitol. 57:331-334(1993).

541. Ribosomal protein S13 signature

Ribosomal protein S13 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S13 is known to be involved in binding fMet-tRNA and, hence, in the initiation of translation. It is a basic protein of 115 to 177 amino-acid residues and belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S13.
 - Plant chloroplast S13 (nuclear encoded). - Red algal chloroplast S13.
 - Cyanelle S13. - Archaebacterial S13. - Plant mitochondrial S13.
 - Mammalian and plant S18.

The best conserved regions in these proteins, located in their C-terminal part have been selected as a signature pattern.

-Consensus pattern: [KRQS]-G-x-R-H-x(2)-[GSNH]-x(2)-[LIVMC]-R-G-Q

[1] Chan Y.-L., Paz V., Wool I.G.
 Biochem. Biophys. Res. Commun. 178:1212-1218(1991).
[2] Otaka E., Hashimoto T., Mizuta K.
 Protein Seq. Data Anal. 5:285-300(1993).

542. Ribosomal protein S14p/S29e (Ribosomal protein S14 signature)

Ribosomal protein S14 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S14 is known to be required for the assembly of 30S particles and may also be responsible for determining the conformation of 16S rRNA at the A site. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups:

- Eubacterial S14.
- Algal and plant chloroplast S14.
- Cyanelle S14.
- Archaebacterial Methanococcus vannielii S14.
- Plant mitochondrial S14.
- Yeast mitochondrial MRP2.
- Mammalian S29.
- Yeast YS29A/B.

S14 is a protein of 53 to 115 amino-acid residues. Our signature pattern is based on the few conserved positions located in the center of these proteins.

Consensus pattern: [RP]-x(0,1)-C-x(11,12)-[LIVMF]-x-[LIVMF]-[SC]-[RG]-x(3)-[RN]

[1] Chan Y.-L., Suzuki K., Olvera J., Wool I.G. Nucleic Acids Res. 21:649-655(1993).
[2] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

543. Ribosomal protein S15 signature

Ribosomal protein S15 is one of the proteins from the small ribosomal subunit. In Escherichia coli, this protein binds to 16S ribosomal RNA and functions at early steps in ribosome assembly. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S15.
- Archaebacterial Halobacterium marismortui HmaS15 (HS11).
- Plant chloroplast S15. - Yeast mitochondrial S28. - Mammalian S13.
- Brugia pahangi and Wuchereria bancrofti S13 (S15). - Yeast S13 (YS15).

S15 is a protein of 80 to 250 amino-acid residues.

A conserved region located in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: [LIVM]-x(2)-H-[LIVMFY]-x(5)-D-x(2)-[SAGN]-x(3)-[LF]-x(9)-[LIVM]-x(2)-[FY]

[1] Dang H., Ellis S.R.
Nucleic Acids Res. 18:6895-6901(1990).

[2] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).

544. Ribosomal protein S16 signature

Ribosomal protein S16 is one of the proteins from the small ribosomal subunit. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups:

- Eubacterial S16.
- Algal and plant chloroplast S16.
- Cyanelle S16.
- Neurospora crassa mitochondrial S24 (cyt-21).

S16 is a protein of about 100 amino-acid residues. A conserved region located in the N-terminal extremity of these proteins has been selected as a signature pattern.

Consensus pattern: [LIVMT]-x-[LIVM]-[KR]-L-[STAK]-R-x-G-[AKR]

[1] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

545. Ribosomal protein S17 signature

Ribosomal protein S17 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S17 is known to bind specifically to the 5' end of 16S ribosomal RNA and is thought to be involved in the recognition of termination codons. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial S17.

- Plant chloroplast S17 (nuclear encoded). - Red algal chloroplast S17.

- Cyanelle S17. - Archaebacterial S17. - Mammalian and plant cytoplasmic S11.
- Yeast S18a and S18b (RP41; YS12).

The best conserved regions located in the C-terminal sections of these proteins have been selected as a signature pattern.

-Consensus pattern: G-D-x-[LIV]-x-[LIVA]-x-[QEK]-x-[RK]-P-[LIV]-S

[1] Gantt J.S., Thompson M.D. J. Biol. Chem. 265:2763-2767(1990).

[2] Herfurth E., Hirano H., Wittmann-Liebold B.
Biol. Chem. Hoppe-Seyler 372:955-961(1991).

[3] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).

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546. Ribosomal protein S17e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrates S17 [1]. - Drosophila S17 [2]. - Neurospora crassa S17 (crp-3).
- Yeast S17a (RP51A) and S17b (RP51B) [3]. - Methanococcus jannaschii MJ0245.

These proteins have from 63 (in archebacteria) to 130 to 146 amino acids and are highly conserved. A region in the central part of these proteins has been selected as a signature.

-Consensus pattern: A-x-I-x-[ST]-K-x-L-R-N-[KR]-I-A-G-[FY]-x-T-H

[1] Chen I.-T., Roufa D.J. Gene 70:107-116(1988).

[2] Maki C., Rhoads D.D., Stewart M.J., van Slyke B., Denell R.E.,
Roufa D.J. Gene 79:289-298(1989).[3] Abovich N., Rosbash M.
Mol. Cell. Biol. 4:1871-1879(1984).

547. Ribosomal protein S18 signature

Ribosomal protein S18 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S18 has been involved in aminoacyl-tRNA binding[1]. It appears to be situated at the tRNA A-site of the ribosome. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities[2], groups: - Eubacterial S18. - Algal and plant chloroplast S18. - Cyanelle S18.As a signature pattern, a conserved region in the central section of the protein has been selected. This region contains two basic residues which may be involved in RNA-binding.-

Consensus pattern: [IV]-[DY]-Y-x(2)-[LIVMT]-x(2)-[LIVM]-x(2)-[FYT]-[LIVM]- [ST]-[DERP]-x-[GY]-K-[LIVM]-x(3)-R-[LIVMAS]-

[1] McDougall J., Choli T., Kruft V., Kapp U., Wittmann-Liebold B. FEBS Lett. 245:253-260(1989).[2] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

548. Ribosomal protein S19 signature

Ribosomal protein S19 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S19 is known to form a complex with S13 that binds strongly to 16S ribosomal RNA. S19 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S19.

- Algal and plant chloroplast S19. - Cyanelle S19. - Archaebacterial S19.
- Plant mitochondrial S19. - Eukaryotic S15 ('rig' protein).

S19 is a protein of 88 to 144 amino-acid residues. Our signature pattern is based on the few conserved positions located in the C-terminal section of these proteins.

-Consensus pattern: [STDNQ]-G-[KRQM]-x(6)-[LIVM]-x(4)-[LIVM]-[GSD]-x(2)-[LF]-[GAS]-[DE]-F-x(2)-[ST]

[1] Kitagawa M., Takasawa S., Kikuchi N., Itoh T., Teraoka H., Yamamoto H., Okamoto H. FEBS Lett. 283:210-214(1991).

[2] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

549. Ribosomal protein S19e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities [1,2]. One of these families consists of: - Mammalian S19. - Drosophila S19.

- Ascaris lumbricoides S19g (ALEP-1) and S19s. - Yeast YS16 (RP55A and RP55B).
- Aspergillus S16. - Halobacterium marismortui HS12.

These proteins have 143 to 155 amino acids.

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A well conserved stretch of 20 residues in the C-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: P-x(6)-[SAN]-x(2)-[LIVMA]-x-R-x-[ALIV]-[LV]-Q-x-L-[EQ]

[1] Etter A., Aboutanos M., Tobler H., Mueller F.
Proc. Natl. Acad. Sci. U.S.A. 88:1593-1596(1991).

[2] Suzuki K., Olvera J., Wool I.G. Biochimie 72:299-302(1990).

550. Ribosomal protein S2 signatures

Ribosomal protein S2 is one of the proteins from the small ribosomal subunit. S2 belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S2. - Algal and plant chloroplast S2.

- Cyanelle S2. - Archaebacterial S2.
- Higher eukaryotes P40 (previously thought to be a laminin receptor).
- Yeast NAB1. - Plant mitochondrial S2. - Yeast mitochondrial MRP4.

S2 is a protein of 235 to 394 amino-acid residues.

Two conserved regions have been selected as signature patterns. One is located in the N-terminal section and the other in the central section.

-Consensus pattern: [LIVMFA]-x(2)-[LIVMFYC](2)-x-[STAC]-[GSTANQEKR]-[STALV]-[HY]-[LIVMF]-G

-Consensus pattern: P-x(2)-[LIVMF](2)-[LIVMS]-x-[GDN]-x(3)-[DENL]-x(3)-[LIVM]-x-E-x(4)-[GNQKRH]-[LIVM]-[AP]

[1] Davis S.C., Tzagoloff A., Ellis S.R.
J. Biol. Chem. 267:5508-5514(1992).

[2] Tohgo A., Takasawa S., Munakata H., Yonekura H., Hayashi N., Okamoto H.
FEBS Lett. 340:133-138(1994).

551. Ribosomal protein S21 signature

Ribosomal protein S21 is one of the proteins from the small ribosomal subunit. So far S21 has only been found in eubacteria. It is a protein of 55 to 70 amino-acid residues. A conserved region in the N-terminal section of the protein has been selected as a signature pattern.

Consensus pattern: [DE]-x-A-[LIY]-[KR]-R-F-K-[KR]-x(3)-[KR]

552. Ribosomal protein S21e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Mammalian S21 [1].

- Caenorhabditis elegans S21 (F37C12.11). - Rice S21 [2].
- Yeast S21 (Ys25) [3]. - Fission yeast S28 [4].

These proteins have 82 to 87 amino acids.

A perfectly conserved nonapeptide in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: L-Y-V-P-R-K-C-S-[SA]

[1] Bhat K.S., Morrison S.G. Nucleic Acids Res. 21:2939-2939(1993).

[2] Nishi R., Hashimoto H., Uchimiya H., Kato A. Biochim. Biophys. Acta 1216:113-114(1993).[3] Suzuki K., Otaka E. Nucleic Acids Res. 16:6223-6223(1988).[4] Itoh T., Okata E., Matsui K.A. Biochemistry 24:7418-7423(1985).

553. Ribosomal protein S24e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Vertebrate S24 [1]. - Yeast Rp50. - Mucor racemosus S24 [2].
- Halobacterium marismortui HS15 [3]. - Methanococcus jannaschii MJ0394.

These proteins have 101 to 148 amino acids.

A well conserved stretch in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: [FYA]-G-x(2)-[KR]-[STA]-x-G-[FY]-[GA]-x-[LIVM]-Y-[DN]-[SDN]

[1] Brown S.J., Jewell A., Maki C.G., Roufa D.J. Gene 91:293-296(1990).

[2] Sosa L., Fonzi W.A., Sypherd P.S. Nucleic Acids Res. 17:9319-9331(1989).[3] Kimura J., Arndt E., Kimura M. FEBS Lett. 224:65-70(1987).

554. Ribosomal protein S26e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of: - Mammalian S26 [1].
- Octopus S26 [2]. - Drosophila S26 (DS31) [3]. - Plant cytoplasmic S26.
- Fungi S26 [4].

These proteins have 114 to 127 amino acids.

A conserved octapeptide in the central part of these proteins has been selected as a signature pattern.

-Consensus pattern: [YH]-C-V-S-C-A-I-H

[1] Kuwano Y., Nakanishi O., Nabeshima Y., Tanaka T., Ogata K.
J. Biochem. 97:983-992(1985).[2] Zinov'eva R.D., Tomarev S.I.
Dokl. Akad. Nauk SSSR 304:464-469(1989).
[3] Itoh N., Ohta K., Ohta M., Kawasaki T., Yamashina I.
Nucleic Acids Res. 17:2121-2121(1989).[4] Wu M., Tan H.
Gene 150:401-402(1994).

555. Ribosomal protein S28e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:
- Mammalian S28 [1]. - Plant S28 [2]. - Fungi S33 [3].
- Methanococcus jannaschii MJ1202.

These proteins have from 64 to 78 amino acids.

A highly conserved nonapeptide from the C-terminal extremity of these proteins has been selected as a signature pattern.

-Consensus pattern: E-[ST]-E-R-E-A-R-x-L

[1] Chan Y.-L., Olvera J., Wool I.G.
Biochem. Biophys. Res. Commun. 179:314-318(1991).
[2] Hwang I., Goodman H.M. Plant Physiol. 102:1357-1358(1993).
[3] Hoekstra R., Ferreira P.M., Bootsman T.C., Mager W.H., Planta R.J.
Yeast 8:949-959(1992).

556. Ribosomal protein S3Ae signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian S3A (was originally known as v-fos transformation effector protein). - Caenorhabditis elegans S3A (F56F3.5).
- Plant cytoplasmic S3A (CYC07) [1]. - Yeast Rp10 (PLC1 and PLC2).
- Fission yeast Rp10 (SpAC13G6.02c). - Methanococcus jannaschii MJ0980.

These proteins have from 220 to 250 amino acids.

A conserved stretch in their N-terminal section was selected as a signature pattern.

-Consensus pattern: [LIV]-x-[GH]-R-[IV]-x-E-x-[SC]-L-x-D-L

[1] Liu J.H., Reid D.M.
Plant Physiol. 109:338-338(1995).

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557. Ribosomal protein S3 signature

Ribosomal protein S3 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S3 is known to be involved in the binding of initiator Met-tRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial S3.

- Algal and plant chloroplast S3. - Cyanelle S3. - Archaebacterial S3.
- Plant mitochondrial S3. - Vertebrate S3. - Insect S3.
- Caenorhabditis elegans S3 (C23G10.3). - Yeast S3 (Rp13).

S3 is a protein of 209 to 559 amino-acid residues.

A conserved region located in the C-terminal section has been selected as a signature pattern.

-Consensus pattern: [GSTA]-[KR]-x(6)-G-x-[LIVMT]-x(2)-[NQSCH]-x(1,3)-[LIVFCA]-x(3)-[LIV]-[DENQ]-x(7)-[LMT]-x(2)-G-x(2)-G

[1] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).

558. Ribosomal protein S4 signature

Ribosomal protein S4 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S4 is known to bind directly to 16S ribosomal RNA. Mutations in S4 have been shown to increase translational error frequencies. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S4. - Algal and plant chloroplast S4.

- Cyanelle S4. - Archaebacterial S4. - Mammalian S9. - Yeast YS11 (SUP45).
- Marchantia polymorpha mitochondrial S4. - Dictyostelium discoideum rp1024.
- Yeast protein NAM9 [3]. NAM9 has been characterized as a suppressor for ochre mutations in mitochondrial DNA. It could be a ribosomal protein that acts as a suppressor by decreasing translation accuracy.

S4 is a protein of 171 to 205 amino-acid residues (except for NAM9 which is much larger). The signature pattern for this protein is based on a conserved region located in the central section of these proteins.

-Consensus pattern: [LIVM]-[DE]-x-R-[LI]-x(3)-[LIVMC]-[VMFYHQ]-[KRT]-x(3)-[STAGCVF]-x-[ST]-x(3)-[SAI]-[KR]-x-[LIVMF](2)

[1] Mizuta K., Hashimoto T., Suzuki K.I., Otaka E.
Nucleic Acids Res. 19:2603-2608(1991).
[2] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).
[3] Boguta M., Dmochowska A., Borsuk P., Wrobel K., Gargouri A., Lazowska J., Slonimski P., Szczesniak B., Kruszewska A.
Mol. Cell. Biol. 12:402-412(1992).

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559. Ribosomal protein S4e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian S4 [1]. Two highly similar isoforms of this protein exist : one coded by a gene on chromosome Y, and the other on chromosome X.
- Plant cytoplasmic S4 [2] - Yeast S7 (YS6). - Archebacterial S4e.

These proteins have 233 to 264 amino acids.

A highly conserved stretch of 15 residues in their N-terminal section has been selected as a signature pattern. Four positions in this region are positively charged residues.

-Consensus pattern: H-x-K-R-[LIVMF]-[SANK]-x-P-x(2)-[WY]-x-[LIVM]-x-[KRP]

[1] Fisher E.M., Beer-Romero P., Brown L.G., Ridley A., McNeil J.A., Lawrence J.B., Willard H.F., Bieber F.R., Page D.C. Cell 63:1205-1218(1990).

[2] Braun H.P., Emmermann M., Mentzel H., Schmitz U.K. Biochim. Biophys. Acta 1218:435-438(1994).

560. Ribosomal protein S5 signature

Ribosomal protein S5 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S5 is known to be important in the assembly and function of the 30S ribosomal subunit. Mutations in S5 have been shown to increase translational error frequencies. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S5.
- Cyanelle S5. - Red algal chloroplast S5. - Archaebacterial S5.
- Mammalian S2 (LLrep3). - Caenorhabditis elegans S2 (C49H3.11).
- Drosophila S2. - Plant S2. - Yeast S4 (SUP44). - Fungi mitochondrial S5.

S5 is a protein of 166 to 254 amino-acid residues. The signature pattern for this protein is based on a conserved region, rich in glycine residues, and located in the N-terminal section of these proteins.

-Consensus pattern: G-[KRQ]-x(3)-[FY]-x-[ACV]-x(2)-[LIVMA]-[LIVM]-[AG]-[DN]-x(2)-G-x-[LIVM]-G-x-[SAG]-x(5,6)-[DEQ]-[LIVMA]-x(2)-A-[LIVMF]

[1] All-Robyn J.A., Brown N., Otaka E., Liebman S.W. Mol. Cell. Biol. 10:6544-6553(1990).[2] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

561. Ribosomal protein S6 signature

Ribosomal protein S6 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S6 is known to bind together with S18 to 16S ribosomal RNA. It belongs to a family of ribosomal proteins which, on the basis of

sequence similarities, groups: - Eubacterial S6. - Red algal chloroplast S6.
- Cyanelle S6.

S6 is a protein of 95 to 208 amino-acid residues. The signature pattern for this protein is based on a conserved region located in the N-terminal section of these proteins.

-Consensus pattern: G-x-[KRC]-[DENQRH]-L-[SA]-Y-x-I-[KRNSA]

562. Ribosomal protein S6e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:
- Mammalian S6 [1]. - Drosophila S6 [2]. - Plant S6 [3]. - Yeast S10 (YS4).
- Halobacterium marismortui HS13 [4]. - Methanococcus jannaschii MJ1260.

S6 is the major substrate of protein kinases in eukaryotic ribosomes [5]; it may have an important role in controlling cell growth and proliferation through the selective translation of particular classes of mRNA.

These proteins have 135 to 249 amino acids.

A conserved stretch of 12 residues in the N-terminal part of these proteins has been selected as a signature pattern.

-Consensus pattern: [LIVM]-[STAMR]-G-G-x-D-x(2)-G-x-P-M

[1] Franco R., Rosenfeld M.G. J. Biol. Chem. 265:4321-4325(1990).

[2] Watson K.L., Konrad K.D., Woods D.F., Bryant P.J.
Proc. Natl. Acad. Sci. U.S.A. 89:11302-11306(1992).

[3] Hansen G., Estruch J.J., Spena A.
Nucleic Acids Res. 20:5230-5230(1992).

[4] Kimura M., Arndt E., Hatakeyama T., Hatakeyama T., Kimura J.
Can. J. Microbiol. 35:195-199(1989).

[5] Bandi H.R., Ferrari S., Krieg J., Meyer H.E., Thomas G.
J. Biol. Chem. 268:4530-4533(1993).

563. Ribosomal protein S7 signature

Ribosomal protein S7 is one of the proteins from the small ribosomal subunit.

In Escherichia coli, S7 is known to bind directly to part of the 3'end of 16S ribosomal RNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2,3], groups: - Eubacterial S7.

- Algal and plant chloroplast S7. - Cyanelle S7. - Archaebacterial S7.
- Plant mitochondrial S7. - Mammalian S5. - Plant S5.
- Caenorhabditis elegans S5 (T05E11.1).

The best conserved region located in the N-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [DENSK]-x-[LIVMDET]-x(3)-[LIVMFTA](2)-x(6)-G-K-[KR]-x(5)-[LIVMF]-[LIVMFC]-x(2)-[STAC]

[1] Klussmann S., Franke P., Bergmann U., Kostka S., Wittmann-Liebold B.
Biol. Chem. Hoppe-Seyler 374:305-312(1993).
[2] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).
[3] Ignatovich O., Cooper M., Kulesza H.M., Beggs J.D.
Nucleic Acids Res. 23:4616-4619(1995).

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564. Ribosomal protein S7e signature

A number of eukaryotic ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:

- Mammalian S7.
- Xenopus S8.
- Insect S7.
- Yeast probable ribosomal protein S7 (N2212).
- Fission yeast probable ribosomal protein S7 (SpAC18G6.13c).

These proteins have about 200 amino acids. A highly conserved stretch of 14 residues which is located in the central section and which is rich in charged residues was selected as a signature pattern.

Consensus pattern: [KR]-L-x-R-E-L-E-K-K-F-[SAP]-x-[KR]-H

[1] Salazar C.E., Mills-Hamm D.M., Kumar V., Collins F.H. Nucleic Acids Res. 21:4147-4147(1993).

565. Ribosomal protein S8 signature

Ribosomal protein S8 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S8 is known to bind directly to 16S ribosomal RNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups: - Eubacterial S8. - Algal and plant chloroplast S8. - Cyanelle S8. - Archaebacterial S8. - Marchantia polymorpha mitochondrial S8. - Mammalian S15A. - Plant S15A. - Yeast S22 (S24).

The best conserved region located in the C-terminal section of these proteins has been selected as a signature pattern.

-Consensus pattern: [GE]-x(2)-[LIV](2)-[STY]-[ST]-x(2)-G-[LIVM](2)-x(4)-[AG]-[KRHAYI]

[1] Otaka E., Hashimoto T., Mizuta K.
Protein Seq. Data Anal. 5:285-300(1993).

566. Ribosomal protein S8e signature

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities [1]. One of these families consists of:

- Mammalian S8. - Caenorhabditis elegans S8 (F42C5.8). - Leishmania major S8.
- Plant S8. - Yeast S8 (S14) (Rp19). - Archebacterial S8e.

These proteins have either about 220 amino acids (in eukaryotes) or about 125 amino acids (in archebacteria). A conserved stretch which is located in the N-terminal section and which is rich in positively charged residues has been selected as a signature pattern.

-Consensus pattern: [KR]-x(2)-[ST]-G-[GA]-x(5)-[HR]-[KG]-[KR]-x-K-x-E-[LM]-G

[1] Engemann S., Herfurth E., Briesemeister U., Wittmann-Liebold B.
J. Protein Chem. 14:189-195(1995).

09555365.061500

567. Ribosomal protein S9 signature

Ribosomal protein S9 is one of the proteins from the small ribosomal subunit. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1,2], groups: - Eubacterial S9. - Algal chloroplast S9.
- Cyanelle S9. - Archaebacterial S9. - Mammalian S16. - Plant S16.
- Yeast mitochondrial ribosomal S9.

A conserved region containing many charged residues and located in the central section of these proteins has been selected as a signature pattern.

-Consensus pattern: G-G-G-x(2)-[GSA]-Q-x(2)-[SA]-x(3)-[GSA]-x-[GSTAV]-[KR]-[GSAL]-[LIF]

[1] Chan Y.-L., Paz V., Olvera J., Wool I.G. FEBS Lett. 263:85-88(1990).

[2] Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

09555326.051500

568. Ribulose-phosphate 3-epimerase family signatures

Ribulose-phosphate 3-epimerase (EC 5.1.3.1) (also known as pentose-5-phosphate 3-epimerase or PPE) is the enzyme that converts D-ribulose 5-phosphate into D-xylulose 5-phosphate in Calvin's reductive pentose phosphate cycle. In Alcaligenes eutrophus two copies of the gene coding for PPE are known [1], one is chromosomally encoded (cbbEC), the other one is on a plasmid (cbbeP). PPE has been found in a wide range of bacteria, archebacteria, fungi and plants. The sequence of PPE is highly related to:

- Escherichia coli D-allulose-6-phosphate 3-epimerase (gene alsE).
- Escherichia coli protein sgcE.
- Mycoplasma genitalium hypothetical protein MG112.

All these proteins have from 209 to 241 amino acid residues.

Two conserved regions which are located respectively in the N-terminal and in the central part of these proteins have been selected as signature patterns.

-Consensus pattern: [LIVMF]-H-[LIVMFY]-D-[LIVM]-x-D-x(1,2)-[FY]-[LIVM]-x-N-x-[STAV]

-Consensus pattern: [LIVMA]-x-[LIVM]-M-[ST]-[VS]-x-P-x(3)-G-Q-x-F-x(6)-[NK]-[LIVMC]

[1] Kusian B., Yoo J.G., Bednarski R., Bowien B.
J. Bacteriol. 174:7337-7344(1992).

569. (Ricin B lectin) Similarity to lectin domain of ricin beta-chain, 3 copies.

This family consists of a triplicated domain involved in cell agglutination in ricin.

570. (Rotamase) PpiC-type peptidyl-prolyl cis-trans isomerase signature
Peptidyl-prolyl cis-trans isomerase (EC 5.2.1.8) (PPIase or rotamase) is an enzyme that accelerates protein folding by catalyzing the cis-trans isomerization of proline imidic peptide bonds in oligopeptides [1]. Most characterized PPiases belong to two families, the cyclophilin-type (see <PDOC00154>) and the the FKBP-type (see <PDOC00426>). Recently a third family has been discovered [2,3]. So far, the only biochemically characterized member of this family is the Escherichia coli protein parvulin (gene ppiC), a small (92 residues) cytoplasmic enzyme that prefers amino acid residues with hydrophobic side chains like leucine and phenylalanine in the P1 position of the peptides substrates. PpiC is evolutionary related to a number of proteins that are also probably PPiases:

- Escherichia coli and Haemophilus influenzae ppiD. PpiD is a PPIase which contains a periplasmic ppiC-like domain anchored to the inner membrane and which seems to be involved in the folding of outer membrane proteins.
- Escherichia coli surA. SurA is a periplasmic protein that contains two ppiC-like domains.
- Nitrogen-assimilating bacteria protein nifM which is involved in the activation and stabilization of the iron-component (nifH) of nitrogenase.
- Bacillus subtilis protein prsA, a membrane-bound lipoprotein involved in protein export.
- Lactococcus and lactobacillus protease maturation protein prtM, a membrane-bound lipoprotein involved in the maturation of a secreted serine

proteinase. - Yeast protein ESS1/PTF1 (processing/termination factor 1).

- Drosophila protein dodo (gene dod). - Mammalian protein PIN1,
- Campylobacter jejuni cell binding factor 2 (CBF2), a secreted antigen.
- Bacillus subtilis hypothetical protein yacD.
- Helicobacter pylori hypothetical protein HP0175.
- A hypothetical slime mold protein.

A conserved region that contains a serine which could play a role in the catalytic mechanism of these enzymes has been selected as a signature pattern.

-Consensus pattern: F-[GSADEI]-x-[LVAQ]-A-x(3)-[ST]-x(3,4)-[STQ]-x(3,5)-[GER]-G-x-[LIVM]-[GS]

[1] Fischer G., Schmid F.X.
Biochemistry 29:2205-2212(1990).

[2] Rudd K.E., Sofia H.J., Koonin E.V., Plunkett G. III, Lazar S., Rouviere P.E. Trends Biochem. Sci. 20:14-15(1995).

[3] Rahfeld J.-U., Ruecknagel K.P., Schelbert B., Ludwig B., Hacker J., Mann K., Fischer G. FEBS Lett. 352:180-184(1994).

09595352.061500

571. (RrnaAD) Ribosomal RNA adenine dimethylases signature

A number of enzymes responsible for the dimethylation of adenosines if ribosomal RNAs (EC 2.1.1.48) have been found [1,2] to be evolutionary related. These enzymes are:

- Bacterial 16S rRNA dimethylase (gene ksgA), which acts in the biogenesis of ribosomes by catalyzing the dimethylation of two adjacent adenosines in the loop of a conserved hairpin near the 3'-end of 16S rRNA. Inactivation of ksgA leads to resistance to the aminoglycoside antibiotic kasugamycin.
- Yeast 18S rRNA dimethylase (gene DIM1), which is functionally similar to ksgA and that dimethylates twin adenosines in the 3'-end of 18S rRNA.
- Bacterial 'erm' methylases. These enzymes confer resistance to macrolide-lincosamide-streptogramin B (MLS) antibiotics - such as erythromycin - by dimethylating the adenine residue at position 2058 of 23S rRNA thus resulting in a reduced affinity between ribosomes and the MLS antibiotics.
- Caenorhabditis elegans hypothetical protein EO2H1.1.

The best conserved regions in these enzymes is located in the N-terminal section and corresponds to a region that is probably involved in S-adenosyl methionine (SAM) binding.

-Consensus pattern: [LIVM]-[LIVMFY]-[DE]-x-G-[STAPV]-G-x-[GA]-x-[LIVMF]-[ST]-x(2)-[LIVM]-x(6)-[LIVMY]-x-[STAGV]-[LIVMFYHC]-E-x-D

[1] van Gemen B., van Knippenberg P.H.
(In) Nucleic acid methylation, Clawson G.A., Willis D.B., Weissbach A., Jones P.A., Eds., pp.19-36, Alan R. Liss Inc, New-York, (1990).
[2] Lafontaine D., Delcour J., Glasser A.L., Desgres J., Vandenhaute J.
J. Mol. Biol. 241:492-497(1994).

09555326.061402

572. (RuBisC0 small) Ribulose bisphosphate carboxylase, small chain. 206 members

573. ATP/GTP-binding site motif A (P-loop) (ras)

From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix. This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5]. There are numerous ATP- or GTP-binding proteins in which the P-loop is found. A number of protein families for which the relevance of the presence of such a motif has been noted are listed below: - ATP synthase alpha and beta subunits. - Myosin heavy chains. - Kinesin heavy chains and kinesin-like proteins. - Dynamins and dynamin-like proteins - Guanylate kinase - Thymidine kinase (- Thymidylate kinase. - Shikimate kinase. - Nitrogenase iron protein family (nifH/frxC) - ATP-binding proteins involved in 'active transport' (ABC transporters) [7] - DNA and RNA helicases [8,9,10]. - GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.). - Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.). - Nuclear protein ran. - ADP-ribosylation factors family - Bacterial dnaA protein - Bacterial recA protein - Bacterial recF protein - Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.). - DNA mismatch repair proteins mutS family - Bacterial type II secretion system protein E. Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the glycolytic kinases. In other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved foradenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.

Consensus pattern: [AG]-x(4)-G-K-[ST]

In addition to the proteins listed above, the 'A' motif is also found in a number of other proteins. Most of these proteins probably bind a nucleotide, but others are definitively not ATP- or GTP-binding (as for example chymotrypsin, or human ferritin light chain).

[1] Walker J.E., Saraste M., Runswick M.J., Gay N.J. EMBO J. 1:945-951(1982).[2] Moller W., Amons R. FEBS Lett. 186:1-7(1985).[3] Fry D.C., Kuby S.A., Mildvan A.S. Proc. Natl. Acad. Sci. U.S.A. 83:907-911(1986).[4] Dever T.E., Glynias M.J., Merrick W.C. Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).[5] Saraste M., Sibbald P.R., Wittinghofer A. Trends Biochem. Sci. 15:430-434(1990).[6] Koonin E.V. J. Mol. Biol. 229:1165-1174(1993).[7] Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P. J. Bioenerg. Biomembr. 22:571-592(1990).[8] Hodgman T.C. Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).[9] Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P. Nature 337:121-122(1989).[10] Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M. Nucleic Acids Res. 17:4713-4730(1989).

GTP-binding nuclear protein ran signature (ras)

Ran (or TC4) is a small abundant nuclear protein that binds and hydrolyzes GTP and which has been implicated in a large number of processes including nucleocytoplasmic transport, RNA synthesis, processing and export and cell cycle checkpoint control [1,2]. Ran is generally included in the RAS 'superfamily' of small GTP-binding proteins [3], but it is only slightly related to the other RAS proteins. It also differs from RAS proteins in that it lacks cysteine residues at its C- terminal and is therefore not subject to prenylation. Instead ran has an acidic C-terminus. It is, however similar to RAS family members in requiring a specific guanine nucleotide exchange factor (GEF) and a specific GTPase activating protein (GAP) as stimulators of overall GTPase activity. The region of the GTP-binding B motif which, in ran, is perfectly conserved has been selected as a signature pattern.

09555225.071502

Consensus pattern: D-T-A-G-Q-E-K-[LF]-G-G-L-R-[DE]-G-Y-Y- Proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop).

[1] Scheffzek K., Klebe C., Fritz-Wolf K., Kabsch W., Wittinghofer A. Nature 374:378-381(1995).[2] Rush M.G., Drivas G., d'Eustachio P. BioEssays 18:103-112(1996).[3] Valencia A., Chardin P., Wittinghofer A., Sander C. Biochemistry 30:4637-4648(1991).

574. recA signature

The bacterial recA protein [1,2,3,E1] is essential for homologous recombination and recombinational repair of DNA damage. RecA has many activities: it filaments, it binds to single- and double-stranded DNA, itbinds and hydrolyzes ATP, it is also a recombinase and, finally, it interacts with lexA causing its activation and leading to its autocatalytic cleavage. RecA is a protein of about 350 amino-acid residues. Its sequence is very well conserved [3,4,5,E1] among eubacterial species. It is also found in the chloroplast of plants [6]. The best conserved region, a nonapeptide located in the middle of the sequence which is part of the monomer-monomer interface in a recA filament has been selected as a signature pattern,.

Consensus pattern: A-L-[KR]-[IF]-[FY]-[STA]-[STAD]-[LIVMQ]-R-

[1] Smith K.C., Wang T.-C. V. BioEssays 10:12-16(1989).[2] Lloyd A.T., Sharp P.M. J. Mol. Evol. 37:399-407(1993).[3] Roca A.I., Cox M.M. Prog. Nucleic Acids Res. Mol. Biol. 56:129-223(1997).[4] Karlin S., Weinstock G.M., Brendel V. J. Bacteriol. 177:6881-6893(1995).[5] Eisen J.A. J. Mol. Evol. 41:1105-1123(1995).[6] Cerutti H.D., Osman M., Grandoni P., Jagendorf A.T. Proc. Natl. Acad. Sci. U.S.A. 89:8068-8072(1992).[E1] http://www.tigr.org/~jeisen/RecA/RecA.html

575. Response regulator receiver domain

This domain receives the signal from the sensor partner inComment: bacterial two-component systems. It is usually found N-terminalComment: to a DNA binding effector domain.

[1] Pao GM, Saier MH; J Mol Evol 1995;40:136-154.

576. Ribonucleotide reductase large subunit signature

*Ribonucleotide reductase (EC 1.17.4.1) [1,2] catalyzes the reductive synthesis of deoxyribonucleotides from their corresponding ribonucleotides. It provides the precursors necessary for DNA synthesis. Ribonucleotide reductase is an oligomeric enzyme composed of a large subunit (700 to 1000 residues) and a small subunit (300 to 400 residues). There are regions of similarities in the sequence of the large chain from prokaryotes, eukaryotes and viruses. One of these regions has been selected as a signature pattern.

Consensus pattern: W-x(2)-[LF]-x(6,7)-G-[LIVM]-[FYRA]-[NH]-x(3)-[STAQLIVM]-[ASC]-x(2)-[PA]-

[1] Nillson O., Lundqvist T., Hahne S., Sjoberg B.-M. Biochem. Soc. Trans. 16:91-94(1988).[2] Reichard P. Science 260:1773-1777(1993).

09595352.021702

577. Ribonuclease T2 family histidine active sites

The fungal ribonucleases T2 from Aspergillus oryzae, M from Aspergillus saitoiand Rh from Rhizopeus niveus are structurally and functionally related 30 Kdglycoproteins [1] that cleave the 3'-5' internucleotide linkage of RNA via a nucleotide 2',3'-cyclic phosphate intermediates (EC 3.1.27.1).A number of other RNAses have been found to be evolutionary related to these fungal enzymes: - Self-incompatibility [2] in flowering plants is often controlled by a single gene (S-gene) that has several alleles. This gene prevents fertilization by self-pollen or by pollen bearing either of the two S- alleles expressed in the style. The self-incompatibility glycoprotein from several higher plants of the solanaceae family has been shown [2,3] to be a ribonuclease. - Phosphate-starvation induced RNAses LE and LX from tomato [4]. These two enzymes are probably involved in a phosphate-starvation rescue system. - Escherichia coli periplasmic RNAse I (EC 3.1.27.6) (gene rna) [5]. - Aeromonas hydrophila periplasmic RNAse. - Haemophilus influenzae hypothetical protein HI0526.Two histidines residues have been shown [6,7] to be involved in the catalytic mechanism of RNase T2 and Rh. These residues and the region around them arehighly conserved in all the sequence described above. Two signature patterns have been developed, one for each of the two active-site histidines. The second pattern also contains a cysteine which is known to be involved in a disulfide bond.

Consensus pattern: [FYWL]-x-[LIVM]-H-G-L-W-P [H is an active site residue]

Consensus pattern: [LIVMF]-x(2)-[HDGTY]-[EQ]-[FYW]-x-[KR]-H-G-x-C [H is an active site residue] [C is involved in a disulfide bond]

[1] Watanabe H., Naitoh A., Suyama Y., Inokuchi N., Shimada H., Koyama T., Ohgi K., Irie M. J. Biochem. 108:303-310(1990).[2] Haring V., Gray J.E., McClure B.A., Anderson M.A., Clarke A.E. Science 250:937-941(1990).[3] McClure B.A., Haring V., Ebert P.R., Anderson M.A., Simpson R.J., Sakiyama F., Clarke A.E. Nature 342:95957(1989).[4] Loeffler A., Glund K., Irie M. Eur. J. Biochem. 214:627-633(1993).[5] Meador J. III, Kennell D. Gene 95:1-7(1990).[6] Kawata Y., Sakiyama F., Hayashi F., Kyogoku Y. Eur. J. Biochem. 187:255-262(1990).[7] Kurihara H., Mitsui Y., Ohgi K., Irie M., Mizuno H., Nakamura K.T. FEBS Lett. 306:189-192(1992).

578. Ribonucleotide reductase large subunit signature. Ribonucleotide reductase (EC 1.17.4.1) [1,2] catalyzes the reductive synthesis of deoxyribonucleotides from their corresponding ribonucleotides. It provides the precursors necessary for DNA synthesis. Ribonucleotide reductase is an oligomeric enzyme composed of a large subunit (700 to 1000 residues) and a small subunit (300 to 400 residues). There are regions of similarities in the sequence of the large chain from prokaryotes, eukaryotes and viruses. One of these regions has been developed as a signature pattern.

Consensus pattern: W-x(2)-[LF]-x(6,7)-G-[LIVM]-[FYRA]-[NH]-x(3)-[STAQLIVM]-[ASC]-x(2)-[PA]-

[1] Nillson O., Lundqvist T., Hahne S., Sjoberg B.-M. Biochem. Soc. Trans. 16:91-94(1988).[2] Reichard P. Science 260:1773-1777(1993).

579. RNase H
RNase H digests the RNA strand of an RNA/DNA hybrid. Important enzyme in retroviral replication cycle, and often found as a domain associated with reverse transcriptases. Structure is a mixed alpha+beta fold with three a/b/a layers.

580. Eukaryotic putative RNA-binding region RNP-1 signature (rrm)

09555652.051401

Many eukaryotic proteins that are known or supposed to bind single-strandedRNA contain one or more copies of a putative RNA-binding domain of about 90amino acids [1,2]. This region has been found in the following proteins: ** Heterogeneous nuclear ribonucleoproteins ** - hnRNP A1 (helix destabilizing protein) (twice). - hnRNP A2/B1 (twice). - hnRNP C (C1/C2) (once). - hnRNP E (UP2) (at least once). - hnRNP G (once). ** Small nuclear ribonucleoproteins ** - U1 snRNP 70 Kd (once). - U1 snRNP A (once). - U2 snRNP B" (once). ** Pre-RNA and mRNA associated proteins ** - Protein synthesis initiation factor 4B (eIF-4B) [3], a protein essential for the binding of mRNA to ribosomes (once). - Nucleolin (4 times). - Yeast single-stranded nucleic acid-binding protein (gene SSB1) (once). - Yeast protein NSR1 (twice). NSR1 is involved in pre-rRNA processing; it specifically binds nuclear localization sequences. - Poly(A) binding protein (PABP) (4 times). ** Others ** - Drosophila sex determination protein Sex-lethal (Sxl) (twice). - Drosophila sex determination protein Transformer-2 (Tra-2) (once). - Drosophila 'elav' protein (3 times), which is probably involved in the RNA metabolism of neurons. - Human paraneoplastic encephalomyelitis antigen HuD (3 times) [4], which is highly similar to elav and which may play a role in neuron-specific RNA processing. - Drosophila 'bicoid' protein (once) [5], a segment-polarity homeobox protein that may also bind to specific mRNAs. - La antigen (once), a protein which may play a role in the transcription of RNA polymerase III. - The 60 Kd Ro protein (once), a putative RNP complex protein. - A maize protein induced by abscisic acid in response to water stress, which seems to be a RNA-binding protein. - Three tobacco proteins, located in the chloroplast [6], which may be involved in splicing and/or processing of chloroplast RNAs (twice). - X16 [7], a mammalian protein which may be involved in RNA processing in relation with cellular proliferation and/or maturation. - Insulin-induced growth response protein Cl-4 from rat (twice). - Nucleolysins TIA-1 and TIAR (3 times) [8] which possesses nucleolytic activity against cytotoxic lymphocyte target cells. may be involved in apoptosis. - Yeast RNA15 protein, which plays a role in mRNA stability and/or poly-(A) tail length [9].Inside the putative RNA-binding domain there are two regions which are highly conserved. The first one is a hydrophobic segment of six residues (which is called the RNP-2 motif), the second one is an octapeptide motif (which is called RNP-1 or RNP-CS). The position of both motifs in the domain is shown in the following schematic representation:

xxxxxxx#####xxxxxxxxxxxxxxxxxxxxxxxxxxxxx#######xxxxxxxxxxxxxxxxxxxxxxxxxx

RNP-2 RNP-1

The RNP-1 motif has been used as a signature pattern for this type of domain.

Consensus pattern: [RK]-G-{EDRKHPCG}-[AGSCI]-[FY]-[LIVA]-x-[FYLM] In most cases the residue in position 3 of the pattern is either Tyr or Phe.

[1] Bandziulis R.J., Swanson M.S., Dreyfuss G. Genes Dev. 3:431-437(1989).[2] Dreyfuss G., Swanson M.S., Pinol-Roma S. Trends Biochem. Sci. 13:86-91(1988).[3] Milburn S.C., Hershey J.W.B., Davies M.V., Kelleher K., Kaufman R.J. EMBO J. 9:2783-2790(1990).[4] Szabo A., Dalmau J., Manley G., Rosenfeld M., Wong E., Henson J., Posner J.B., Furneaux H.M. Cell 67:325-333(1991).[5] Rebagliati M. Cell 58:231-232(1989).[6] Li Y., Sugiura M. EMBO J. 9:3059-3066(1990).[7] Ayane M., Preuss U., Koehler G., Nielsen P.J. Nucleic Acids Res. 19:1273-1278(1991).[8] Kawakami A., Tian Q., Duan X., Streuli M., Schlossman S.F., Anderson P. Proc. Natl. Acad. Sci. U.S.A. 89:8681-8685(1992).[9] Minvielle-Sebastia L., Winsor B., Bonneaud N., Lacroute F. Mol. Cell. Biol. 11:3075-3087(1991).

09555326.061502

581. Rubredoxin signature

Rubredoxins [1] are small electron-transfer prokaryotic proteins. They contain an iron atom which is ligated by four cysteine residues. Rubredoxins are, in some cases, functionally interchangeable with ferredoxins.

A conserved region that includes two of the cysteine residues that bind the iron atom has been selected as a pattern for these proteins.

Consensus pattern: [LIVM]-x(3)-W-x-C-P-x-C-[AGD] [The two C's bind the iron atom]

In Pseudomonas oleovorans rubredoxin 2 (gene alkG) [2], this pattern is found twice because alkG has two rubredoxin domains.

Rubrerythrin [3], a protein with inorganic pyrophosphatase activity from Desulfovibrio vulgaris possesses a C-terminal rubredoxin-like domain, but this domain is too divergent to be detected by the above pattern.

[1] Berg J.M., Holm R.H.(In) Iron-sulfur proteins, Spiro T.G., Ed., pp1-66, Wiley, New-York, (1982). [2] Kok M., Oldenhuis R., der Linden M.P.G., Meulenberg C.H.C.,

Kingma J., Witholt B., J. Biol. Chem. 264:5442-5451(1989). [3] van Beeumen J.J., van Driessche G., Liu M.-Y., Le Gall J., J. Biol. Chem. 266:20645-20653(1991).

582. (rvp) Eukaryotic and viral aspartyl proteases active site

Aspartyl proteases, also known as acid proteases, (EC 3.4.23.-) are a widely distributed family of proteolytic enzymes [1,2,3] known to exist invertebrates, fungi, plants, retroviruses and some plant viruses. Aspartate proteases of eukaryotes are monomeric enzymes which consist of two domains. Each domain contains an active site centered on a catalytic aspartyl residue. The two domains most probably evolved from the duplication of an ancestral gene encoding a primordial domain. Currently known eukaryotic aspartyl proteases are: - Vertebrate gastric pepsins A and C (also known as gastricsin). - Vertebrate chymosin (rennin), involved in digestion and used for making cheese. - Vertebrate lysosomal cathepsins D (EC 3.4.23.5) and E (EC 3.4.23.34). - Mammalian renin (EC 3.4.23.15) whose function is to generate angiotensin I from angiotensinogen in the plasma. - Fungal proteases such as aspergillopepsin A (EC 3.4.23.18), candidapepsin (EC 3.4.23.24), mucoropepsin (EC 3.4.23.23) (mucor rennin), endothiapepsin (EC 3.4.23.22), polyporopepsin (EC 3.4.23.29), and rhizopuspepsin (EC 3.4.23.21). - Yeast saccharopepsin (EC 3.4.23.25) (proteinase A) (gene PEP4). PEP4 is implicated in posttranslational regulation of vacuolar hydrolases. - Yeast barrier pepsin (EC 3.4.23.35) (gene BAR1); a protease that cleaves alpha-factor and thus acts as an antagonist of the mating pheromone. - Fission yeast sxa1 which is involved in degrading or processing the mating pheromones. Most retroviruses and some plant viruses, such as badnaviruses, encode for anaspartyl protease which is an homodimer of a chain of about 95 to 125 amino acids. In most retroviruses, the protease is encoded as a segment of a polyprotein which is cleaved during the maturation process of the virus. It is generally part of the pol polyprotein and, more rarely, of the gagpolyprotein. Conservation of the sequence around the two aspartates of eukaryotic aspartyl proteases and around the single active site of the viral proteases allows us to develop a single signature pattern for both groups of protease. Consensus pattern: [LIVMFGAC]-[LIVMTADN]-[LIVFSA]-D-[ST]-G-[STAV]-[STAPDENQ]- x-[LIVMFSTNC]-x-[LIVMFGTA] [D is the active site residue] – [1] Foltmann B. Essays Biochem. 17:52-84(1981).[2] Davies D.R. Annu. Rev. Biophys. Chem. 19:189-215(1990).[3] Rao J.K.M., Erickson J.W., Wlodawer A. Biochemistry 30:4663-4671(1991).[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:105-120(1995).

583. (rvt) Reverse transcriptase (RNA-dependent DNA polymerase)

A reverse transcriptase gene is usually indicative of a mobile element such as a retrotransposon or retrovirus. Reverse transcriptases occur in a variety of mobile elements, including retrotransposons, retroviruses, group II introns, bacterial msDNAs, hepadnaviruses, and caulimoviruses. Number of members: 1233

[1] Medline: 91006031. Origin and evolution of retroelements based upon their reverse transcriptase sequences. Xiong Y, Eickbush TH; EMBO J 1990;9:3353-3362.

09555329.061502

584. (S-AdoMet synt) S-adenosylmethionine synthetase signatures

S-adenosylmethionine synthetase (EC <u>2.5.1.6</u>) is the enzyme that catalyzes theformation of S-adenosylmethionine (AdoMet) from methionine and ATP [1]. AdoMet is an important methyl donor for transmethylation and is also the propylamino donor in polyamine biosynthesis. In bacteria there is a single isoform of AdoMet synthetase (gene metK), there are two in budding yeast (genes SAM1 and SAM2) and in mammals while in plants there is generally a multigene family.The sequence of AdoMet synthetase is highly conserved throughout isozymes and species. Two signature patterns have been selected for this type of enzyme; the first is a hexapeptide which seems to be involved in ATP-binding; the second is an almost perfectly conserved glycine-rich nonapeptide.

Consensus pattern: G-A-G-D-Q-G-x(3)-G-[FYH]-Sequences known to belong to this class detected by the pattern:

Consensus pattern: G-[GA]-G-[ASC]-F-S-x-K-[DE]

[1] Horikawa S., Sasuga J., Shimizu K., Ozasa H., Tsukada K. J. Biol. Chem. 265:13683-13686(1990).

585. S1 RNA binding domain

The S1 domain occurs in a wide range of RNAComment: associated proteins. It is structurally similarComment: to cold shock protein which binds nucleic acids.Comment: The S1 domain has an OB-fold structure.

[1] Bycroft M, Hubbard TJ, Proctor M, Freund SM, Murzin AG; Cell 1997;88:235-242.

586. SAICAR synthetase signatures

Phosphoribosylaminoimidazole-succinocarboxamide synthase (EC 6.3.2.6) (SAICARsynthetase) catalyzes the seventh step in the de novo purine biosynthetic pathway; the ATP-dependent conversion of 5'-phosphoribosyl-5-aminoimidazole-4-carboxylic acid and aspartic acid to SAICAR [1]. In bacteria (gene purC),fungi (gene ADE1) and plants, SAICAR synthetase is a monofunctional protein;in higher vertebrates it is the N-terminal domain of a bifunctional enzyme that also catalyze phosphoribosylaminoimidazole carboxylase (AIRC) activity. Two conserved regions in the central section of this enzyme have been selected as signature patterns for SAICAR synthetase.

Consensus pattern: [LIVMF](2)-P-[LIVM]-E-x-[LIVM]-[LIVMCA]-R-x(3)-[TA]-G-S-

Consensus pattern: [LIVM]-[LIVMA]-D-x-K-[LIVMFY]-E-F-G

[1] Zalkin H., Dixon J.E. Prog. Nucleic Acid Res. Mol. Biol. 42:259-287(1992).

587. (SCP) Extracellular proteins SCP/Tpx-1/Ag5/PR-1/Sc7 signatures

A variety of extracellular proteins from eukaryotes have been found to be evolutionary related: - Rodent sperm-coating glycoprotein (SCP), also known as acidic epididymal glycoprotein (AEG) . This protein is thought to be involved in sperm maturation [1]. It is a protein of about 220 residues and probably contains eight disulfide bonds. - Mammalian testis-specific protein Tpx-1 [2]. Tpx-1 is highly related to SCP's. - Mammalian glioma pathogenesis-related protein (GliPR). - Lizard helothermine, a toxin that blocks ryanodine receptors. - Venom allergen 5 (Ag5) from vespid wasps and venom allergen 3 (Ag3) from fire ants. These proteins are potent allergens and are the main cause of allergic reactions to stings from insects of the hymenoptera family [3]. Ag5/3 are proteins of about 200 residues and contain four disulfide bonds. - Plant pathogenesis proteins of the PR-1 family [4]. These proteins are synthesized during pathogen infection or other stress-related responses. They are proteins of about 130 to 140 residues and probably contain three disulfide bonds. - Proteins Sc7 and Sc14 from the basidomycete fungus Schizophyllum commune. These extracellular proteins are loosely associated with fruit body hyphal walls [5]. Sc7/14 are proteins of about 180 residues and probably contain two disulfide bonds. - Ancylostoma secreted protein from dog hookworm. - Yeast hypothetical proteins YJL078c, YJL079c and YKR013w.The exact function of these proteins is not yet known. Two conserved regions located in their C-terminal half have been selected as signature patterns. The second signature contains a cysteine which is known to be involved in a disulfide bond in Ag5.

Consensus pattern: [GDER]-H-[FYWH]-T-Q-[LIVM](2)-W-x(2)-[STN]

Consensus pattern: [LIVMFYH]-[LIVMFY]-x-C-[NQRHS]-Y-x-[PARH]-x-[GL]-N-[LIVMFYWDN] [C is involved in a disulfide bond]

[1] Mizuki N., Kasahara M. Mol. Cell. Endocrinol. 89:25-32(1992).[2] Kasahara M., Gutknecht J., Brew K., Spurr N., Goodfellow P.N. Genomics 5:527-534(1989).[3] Lu G., Villalba M., Coscia M.R., Hoffman D.R., King T.P. J. Immunol. 150:2823-2830(1993).[4] Dixon D.C., Cutt J.R., Klessig D.F. EMBO J. 10:1317-1324(1991).[5] Schuren F.H.J., Asgeirsdottir S.A., Kothe E.M., Scheer J.M.J., Wessels J.G.H. J. Gen. Microbiol. 139:2083-2090(1993).

588. SET domain

SET domains appear to be protein-protein interactionComment: domains. It has been demonstrated that SET domainsComment: mediate interactions with a family of proteins thatComment: display similarity with dual-specificity phosphatasesComment: (dsPTPases) [2].

[1] Tripoulas N, LaJeunesse D, Gildea J, Shearn A; Genetics 1996;143:913-928. [2] Cui X, De Vivo I, Slany R, Miyamoto A, Firestein R, Cleary, ML; Nat Genet 1998;18:331-337.

589. Src homology 3 (SH3) domain profile

The Src homology 3 (SH3) domain is a small protein domain of about 60 amino-acid residues first identified as a conserved sequence in the non-catalytic part of several cytoplasmic protein tyrosine kinases (e.g. Src, Abl, Lck) [1].Since then, it has been found in a great variety of other intracellular or membrane-associated proteins [2,3,4,5].The SH3 domain has a characteristic fold which consists of five or six beta-strands arranged as two tightly packed anti-parallel beta sheets. The linker regions may contain short helices [6].The function of the SH3 domain is not well understood. The current opinion is that they mediate assembly of specific protein complexes via binding to proline-rich peptides [7].In general SH3 domains are found as single copies in a given protein, but there is a significant number of protein with two SH3 domains and a few with 3 or 4 copies. So far, SH3 domains have been identified in the following proteins: - Many vertebrate, invertebrate and retroviral cytoplasmic (non-receptor) protein tyrosine kinases. In particular in the Src, Abl, Bkt, Csk and ZAP70 families of kinases. - Mammalian phosphatidylinositol-specific phospholipase C-gamma-1 and -2. - Mammalian phosphatidyl inositol 3-kinase regulatory p85 subunit. - Mammalian Ras GTPase-activating protein (GAP). - Adaptor proteins mediating binding of guanine nucleotide exchange factors to growth factor receptors: vertebrate GRB2, Caenorhabditis elegans sem-5 and Drosophila DRK. All of which have two SH3 domains. - Mammalian Vav oncoprotein, a guanine nucleotide exchange factor of the CDC24 family. - Some guanine-nucleotide releasing factors of the CDC25 family: yeast CDC25, yeast SCD25, fission yeast ste6. - MAGUK proteins. These proteins consist of at least three types of domains: one or more copies of the DHR domain, a SH3 domain and a C-terminal guanylate kinase domain. Members of this family are: Drosophila lethal(1)discs large-1 tumor suppressor protein (gene Dlg1), mammalian tight junction protein ZO-1, vertebrate erythrocyte membrane protein p55, Caenorhabditis elegans protein lin-2, rat protein CASK and mammalian synaptic proteins SAP90/PSD-95, CHAPSYN-110/PSD-93, SAP97/DLG1 and SAP102. - Miscellanous proteins interacting with vertebrate receptor protein tyrosine kinases: mammalian cytoplasmic protein Nck (3 copies), oncoprotein Crk (2 copies). - Chicken Src substrate p80/85 protein (cortactin) and the similar human hemopoietic lineage cell specific protein Hs1. - Mammalian dihydrouridine-sensitive L-type calcium channel beta (regulatory) subunit including the related human myasthenic syndrome antigen B (MSYB). - Mammalian neutrophil cytosolic activators of NADPH oxidase: p47 (NCF-1), p67 (NCF-2), and a potential homolog from Caenorhabditis elegans (B0303.7). NCF-1 and -2 have two copies of the SH3 domain, while B0303.7 has four. - Some myosin heavy chains from amoebae, slime molds and yeast (gene MYO3). - Vertebrate and Drosophila spectrin and fodrin alpha-chain. - Human amphiphysin. - Yeast actin-binding protein ABP1. - Yeast actin-binding protein SLA1 (3 copies). - Yeast protein BEM1 and the fission yeast homolog scd2 (or ral3) (2 copies). - Yeast BEM1-binding proteins BOI2 (BEB1) and BOB1 (BOI1). - Yeast fusion protein FUS1. - Yeast protein RSV167. - Yeast protein SSU81. - Yeast hypothetical proteins YAR014c (1 copy), YFR024c (1 copy), YHL002w (1 copy), YHR016c (1 copy), YJL020C (1 copy), YHR114w (2 copies) and the fission yeast homolog SpAC12C2.05c. - Caenorhabditis elegans hypothetical proteins F42H10.3. The profile developed to detect SH3 domains is based on a structural alignment consisting of 5 gap-free blocks and 4 linker regions totaling 62 match positions.

[1] Mayer B.J., Hamaguchi M., Hanafusa H. Nature 332:272-275(1988).[2] Musacchio A., Gibson T., Lehto V.P., Saraste M. FEBS Lett. 307:55-61(1992).[3] Pawson T., Schlessinger J. Curr. Biol. 3:434-442(1993).[4] Mayer B.J., Baltimore D. Trends Cell Biol. 3:8-13(1993).[5] Pawson T. Nature 373:573-580(1995).[6] Kuriyan J., Cowburn D. Curr. Opin. Struct. Biol. 3:828-837(1993).[7] Morton C.J., Campbell I.D. Curr. Biol. 4:615-617(1994).

590. Serine hydroxymethyltransferase pyridoxal-phosphate attachment site (SHMT)

Serine hydroxymethyltransferase (EC 2.1.2.1) (SHMT) [1] catalyzes the transfer of the hydroxymethyl group of serine to tetrahydrofolate to form 5,10-methylenetetrahydrofolate and glycine. In vertebrates, it exists in acytoplasmic and a mitochondrial form whereas only one form is found in prokaryotes. Serine hydroxymethyltransferase is a pyridoxal-phosphate containing enzyme. The pyridoxal-P group is attached to a lysine residue around which the sequence is highly conserved in all forms of the enzyme.

Consensus pattern: [DEH]-[LIVMFY]-x-[STMV]-[GST]-[ST](2)-H-K-[ST]-[LF]-x-G-[PAC]-[RQ]-[GSA]-[GA] [K is the pyridoxal-P attachment site]

[1] Usha R., Savithri H.S., Rao N.A. Biochim. Biophys. Acta 1204:75-83(1994).

591. SIS domain

SIS (Sugar ISomerase) domains are found in many phosphosugar isomerases and phosphosugar binding proteins.

[1] Teplyakov A, Obmolova G, Badet-Denisot MA, Badet B, Polikarpov I; Structure 1998;6:1047-1055.

592. (SKI) Shikimate kinase signature

Shikimate kinase (EC 2.7.1.71) catalyzes the fifth step in the biosynthesis from chorismate of the aromatic amino acids (the shikimate pathway) inbacteria (gene aroK or aroL), plants and in fungi (where it is part of a multifunctional enzyme which catalyzes five consecutive steps in this pathway).Shikimate kinase is a small protein of about 200 residues. A conserved region that contains a run of three glycines has been selected as a signature pattern. Consensus pattern: [KR]-x(2)-E-x(3)-[LIVMF]-x(8,12)-[LIVMF](2)-[SA]-x-G(3)- x-[LIVMF]. Proteins belonging to this family also contain a copy of the ATP/GTP- binding motif 'A' (P-loop).

593. SNAP-25 family

SNAP-25 (synaptosome-associated protein 25 kDa) proteins are components of SNARE complexes. Members of this family contain a cluster of cysteine residues that can be palmitoylated for membrane attachment [2].

[1]Brennwald P, Kearns B, Champion K, Keranen S, Bankaitis V, Novick P; Cell 1994;79:245-258. [2] Risinger C, Blomqvist AG, Lundell I, Lambertsson A, Nassel D, Pieribone VA, Brodin L, Larhammar D; J Biol Chem 1993;268:24408-24414.

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594. SNF2 and others N-terminal domain

This domain is found in proteins involved in a variety of processes including transcription regulation (e.g., SNF2, STH1, brahma, MOT1) , DNA repair (e.g., ERCC6, RAD16, RAD5), DNA recombination (e.g., RAD54), and chromatin unwinding (e.g., ISWI) as well as a variety of other proteins with little functional information (e.g., lodestar, ETL1).

595. Staphylococcal nuclease homologues (Snase)
Present in all three domains of cellular life. Four copies in the transcriptional coactivator p100. These, however, appear to lack the active site residues of Staphylococcal nuclease. Positions 14 (Asp-21), 34 (Arg-35), 39 (Asp-40), 42 (Glu-43) andComment: 110 (Arg-87) [SNase numbering in parentheses] are thought to be involved in substrate-binding and catalysis.

[1] Ponting CP; Protein Sci 1997;6:459-463. [2] Callebaut I, Mornon JP; Biochem J 1997;321:125-132.

596. SPRY domainA

SPRY Domain is named from SPla and the RYanodine Receptor. Domain of unknown function. Distant homologues are domains in Comment: butyrophilin/marenostrin/pyrin homologues.

[1] Ponting C, Schultz J, Bork P; Trends Biochem Sci 1997;22:193-194.

09595325.061502

597. (SQS PSY) Squalene and phytoene synthases signatures

Two different polyisoprene synthases have been shown [1,2,3] to share a number of regions of sequence similarities: - Squalene synthase (EC 2.5.1.21) (farnesyl-diphosphate farnesyltransferase) (SQS), which catalyzes the conversion of two molecules of farnesyl diphosphate (FPP) into squalene. It is the first committed step in the cholesterol biosynthetic pathway. The reaction carried out by SQS is catalyzed in two separate steps: the first is a head-to-head condensation of the two molecules of FPP to form presqualene diphosphate; this intermediate is then rearranged in a NADP-dependent reduction, to form squalene. SQS is found in eukaryotes. In yeast it is encoded by the ERG9 gene, in mammals by the FDFT1 gene. SQS seems to be membrane-bound. - Phytoene synthase (EC 2.5.1.-) (PSY), which catalyzes the conversion of two molecules of geranylgeranyl diphosphate (GGPP) into phytoene. It is the second step in the biosynthesis of carotenoids from isopentenyl diphosphate. The reaction carried out by PSY is catalyzed in two separate steps: the first is a head-to-head condensation of the two molecules of GGPP to form prephytoene diphosphate; this intermediate is then rearranged to form phytoene. PSY is found in all organisms that synthesize carotenoids: plants and photosynthetic bacteria as well as some non-photosynthetic bacteria and fungi. In bacteria PSY is encoded by the gene crtB. In plants PSY is localized in the chloroplast. As it can be seen from the description above, both SQS and PSY share a number of functional similarities which are also reflected at the level of their primary structure. In particular three well conserved regions are shared bySQS and PSY; they could be involved in substrate binding and/or the catalytic mechanism. Signature patterns have been developed for the second and third conserved regions; they are localized in the central part of these enzymes.

Consensus pattern: Y-[CSAM]-x(2)-[VSG]-A-[GSA]-[LIVAT]-[IV]-G-x(2)-[LMSC]- x(2)-[LIV]

Consensus pattern: [LIVM]-G-x(3)-Q-x(2,3)-N-[IF]-x-R-D-[LIVMFY]-x(2)-[DE]- x(4,7)-R-x-[FY]-x-P-

[1] Summers C., Karst F., Charles A.D. Gene 136:185-192(1993).[2] Robinson G.W., Tsay Y.H., Kienzle B.K., Smith-Monroy C.A., Bishop R.W. Mol. Cell. Biol. 13:2706-2727(1993).[3] Roemer S., Hugueney P., Bouvier F., Camara B., Kuntz M. Biochem. Biophys. Res. Commun. 196:1414-1421(1993).

598. SRP54-type proteins GTP-binding domain signature

The signal recognition particle (SRP) is an oligomeric complex that mediates targeting and insertion of the signal sequence of exported proteins into the membrane of the endoplasmic reticulum. SRP consists of a 7S RNA and six protein subunits. One of these subunits, the 54 Kd protein (SRP54), is a GTP-binding protein that interacts with the signal sequence when it emerges from the ribosome. The N-terminal 300 residues of SRP54 include the GTP-binding site (G-domain) and are evolutionary related to similar domains in other proteins which are listed below [1]. - Escherichia coli and Bacillus subtilis ffh protein (P48), a protein which seems to be the prokaryotic counterpart of SRP54. Ffh is associated with a 4.5S RNA in the prokaryotic SRP complex. - Signal recognition particle receptor alpha subunit (docking protein), an integral membrane GTP-binding protein which ensures, in conjunction with SRP, the correct targeting of nascent secretory proteins to the endoplasmic reticulum membrane. The G-domain is located at the C-terminal extremity of the protein. - Bacterial ftsY protein, a protein which is believed to play a similar role to that of the docking protein in eukaryotes. The G-domain is located at the C-terminal extremity of the protein. - The pilA protein from Neisseria gonorrhoeae which seems to be the homolog of ftsY. - A protein from the archaebacteria Sulfolobus solfataricus. This protein is also believed to be a docking protein. The G-domain is also at the C- terminus. - Bacterial flagellar biosynthesis protein flhF. The best conserved regions in those domains are the sequence motifs that are part of the GTP-binding site, but as those regions are not specific to these proteins, they were not used as a signature pattern. Instead, a conserved region located at the C-terminal end of the domain was selected.

Consensus pattern: P-[LIVM]-x-[FYL]-[LIVMAT]-[GS]-x-[GS]-[EQ]-x(4)-[LIVMF]

[1] Althoff S., Selinger D., Wise J.A. Nucleic Acids Res. 22:1933-1947(1994).

599. (STphosphatase) Serine/threonine specific protein phosphatases signature

Serine/threonine specific protein phosphatases (EC 3.1.3.16) (PP) [1,2,3] are enzymes that catalyze the removal of a phosphate group attached to a serine or evolutionary related. - Protein phosphatase-1 (PP1) is an enzyme of broad specificity. It is inhibited by two thermostable proteins, inhibitor-1 and -2. In mammals, there are two closely related isoforms of PP-1: PP-1alpha and PP-1beta, produced by alternative splicing of the same gene. In Emericella nidulans, PP-1 (gene bimG) plays an important role in mitosis control by reversing the action of the nimA kinase. In yeast, PP-1 (gene SIT4) is involved in dephosphorylating the large subunit of RNA polymerase II. - Protein phosphatase-2A (PP2A) is also an enzyme of broad specificity. PP2A is a trimeric enzyme that consist of a core composed of a catalytic subunit associated with a 65 Kd regulatory subunit and a third variable subunit. In mammals, there are two closely related isoforms of the catalytic subunit of PP2A: PP2A-alpha and PP2A-beta, encoded by separate genes. - Protein phosphatase-2B (PP2B or calcineurin), a calcium-dependent enzyme whose activity is stimulated by calmodulin. It is composed of two subunits: the catalytic A-subunit and the calcium-binding B-subunit. The specificity of PP2B is restricted.In addition to the above-mentioned enzymes, some additional serine/threoninespecific protein phosphatases have been characterized and are listed below. - Mammalian phosphatase-X (PP-X), and Drosophila phosphatase-V (PP-V) which are closely related but yet distinct from PP2A. - Yeast phosphatase PPH3, which is similar to PP2A, but with different enzymatic properties. - Drosophila phosphatase-Y (PP-Y), and yeast phosphatases Z1 and Z2 (genes PPZ1 and PPZ2) which are closely related but yet distinct from PP1. - Drosophila retinal degeneration protein C (gene rdgC), a calcium-binding phosphatase required to prevent light-induced retinal degeneration. - Phages Lambda and Phi-80 ORF-221 which have been shown to have phosphatase activity and are related to mammalian PP's. The best conserved regions in these proteins is a perfectly conserved pentapeptide that can be used as a signature pattern.

Consensus pattern: [LIVM]-R-G-N-H-E-

[1] Cohen P. Annu. Rev. Biochem. 58:453-508(1989).[2] Cohen P., Cohen P.T.W. J. Biol. Chem. 264:21435-21438(1989).[3] Cohen P.T.W., Brewis N.D., Hughes V., Mann D.J. FEBS Lett. 268:355-359(1990).

600. Translation initiation factor SUI1 signature

In budding yeast (Saccharomyces cerevisiae), SUI1 is a translation initiation factor that functions in concert with eIF-2 and the initiator tRNA-Met in directing the ribosome to the proper start site of translation [1]. SUI1 is a protein of 108 residues. Close homologs of SUI1 have been found [2] in mammals, insects and plants. SUI1 is also evolutionary related to hypothetical proteins from Escherichia coli (yciH), Haemophilus influenzae (HI1225) and Methanococcus vannielii. A conserved region in the C-terminal section has been selected as a signature pattern.

Consensus pattern: [LIVM]-[EQ]-[LIVM]-Q-G-[DEN]-[KHQ]-[KRV]

[1] Yoon H., Donahue T.F. Mol. Cell. Biol. 12:248-260(1992).[2] Fields C.A., Adams M.D. Biochem. Biophys. Res. Commun. 198:288-291(1994).

601. (S T dehydratase) Serine/threonine dehydratases pyridoxal-phosphate attachment site

Serine and threonine dehydratases [1,2] are functionally and structurally related pyridoxal-phosphate dependent enzymes: - L-serine dehydratase (EC <u>4.2.1.13</u>) and D-serine dehydratase (EC <u>4.2.1.14</u>) catalyze the dehydratation of L-serine (respectively D-serine) into ammonia and pyruvate. - Threonine dehydratase (EC <u>4.2.1.16</u>) (TDH) catalyzes the dehydratation of threonine into alpha-ketobutarate and ammonia. In Escherichia coli and other microorganisms, two classes of TDH are known to exist. One is involved in the biosynthesis of isoleucine, the other in hydroxamino acid catabolism.Threonine synthase (EC <u>4.2.99.2</u>) is also a pyridoxal-phosphate enzyme, it catalyzes the transformation of homoserine-phosphate into threonine. It has been shown [3] that threonine synthase is distantly related to the serine/threonine dehydratases. In all these enzymes, the pyridoxal-phosphate group is attached to a lysine residue. The sequence around this residue is sufficiently conserved to allow the derivation of a pattern specific to serine/threonine dehydratases and threonine synthases.

Consensus pattern: [DESH]-x(4,5)-[STVG]-x-[AS]-[FYI]-K-[DLIFSA]-[RVMF]-[GA]-[LIVMGA] [The K is the pyridoxal-P attachment site]

[1] Ogawa H., Gomi T., Konishi K., Date T., Naakashima H., Nose K., Matsuda Y., Peraino C., Pitot H.C., Fujioka M. J. Biol. Chem. 264:15818-15823(1989).[2] Datta P., Goss T.J., Omnaas J.R., Patil R.V. Proc. Natl. Acad. Sci. U.S.A. 84:393-397(1987).[3] Parsot C. EMBO J. 5:3013-3019(1986).[4] Grabowski R., Hofmeister A.E.M., Buckel W. Trends Biochem. Sci. 18:297-300(1993).

Cysteine synthase/cystathionine beta-synthase P-phosphate attachment site

Cysteine synthase (CSase) is the pyridoxal-phosphate dependent enzyme responsible [1] for the formation of cysteine from O-acetyl-serine and hydrogen sulfide with the concomitant release of acetic acid. In bacteria suchas Escherichia coli, two forms of the enzyme are known (genes cysK and cysM).In plants there are also two forms, one located in the cytoplasm and the otherin chloroplasts.Cystathionine beta-synthase [2] catalyzes the first irreversiblestep in homocysteine transulfuration; the conjugation of homocysteine andserine forming cystathionine. Like Csase it is a pyridoxal-phosphate dependent enzyme. The two types of enzymes are evolutionary related. The pyridoxal-phosphategroup of CSases has been shown to be attached to a lysine residue which is located in the N-terminal section of these enzymes; the sequence around this residue is highly conserved and can be used as a signature pattern to detect this class of enzymes.

Consensus pattern: K-x-E-x(3)-[PA]-[STAGC]-x-S-[IVAP]-K-x-R-x-[STAG]-x(2)- [LIVM] [The 2nd K is the pyridoxal-P attachment site

[1] Saito K., Kurosawa M., Murakoshi I. FEBS Lett. 328:111-114(1993).[2] Swaroop M., Bradley K., Ohura T., Tahara T., Roper M.D., Rosenberg L.E., Kraus J.P. J. Biol. Chem. 267:11455-11461(1992).

602. S locus glycop

S-locus glycoprotein family. In Brassicaceae, self-incompatible plants have a self/non-self Comment: recognition system. This is sporophytically controlled by Comment: multiple alleles at a single locus (S). S-locus glycoproteins,Comment: as well as S-receptor kinases, are in linkage with the S-alleles [1].Number of members: 128

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[1] Evolutionary aspects of the S-related genes of the Brassica self-incompatibility system: synonymous and nonsynonymous base substitutions. Hinata K, Watanabe M, Yamakawa S, Satta Y, Isogai A; Genetics 1995;140:1099-1104. [2] Polymorphism of the S-locus glycoprotein gene (SLG) and the S-locus related gene (SLR1) in Raphanus sativus L. and self-incompatible ornamental plants in the Brassicaceae. Sakamoto K, Kusaba M, Nishio T; Mol Gen Genet 1998;258:397-403.

603. (sdh cyt) Succinate dehydrogenase cytochrome b subunit signatures

Succinate dehydrogenase (SDH) is a membrane-bound complex of two main components: a membrane-extrinsic component composed of an FAD-binding flavoprotein and an iron-sulfur protein, and a hydrophobic component composed of a cytochrome B and a membrane anchor protein. The cytochrome b component is a mono heme transmembrane protein [1,2,3] belonging to a family that groups: - Cytochrome b-556 from bacterial SDH (gene sdhC). - Cytochrome b560 from the mammalian mitochondrial SDH complex. - Cytochrome b560 subunit encoded in the mitochondrial genome of some algae and in the plant Marchantia polymorpha. - Cytochrome b from yeast mitochondrial SDH complex (gene SDH3 or CYB3). - Protein cyt-1 from Caenorhabditis.These cytochromes are proteins of about 130 residues that comprise threetransmembrane regions. There are two conserved histidines which may beinvolved in binding the heme group. Two signature patterns have been developed that include these histidine residues.

Consensus pattern: R-P-[LIVMT]-x(3)-[LIVM]-x(6)-[LIVMWPK]-x(4)-S-x(2)-H-R-x- [ST] [H could be a heme ligand]

Consensus pattern: H-x(3)-[GA]-[LIVMT]-R-[HF]-[LIVMF]-x-[FYWM]-D-x-[GVA] [H could be a heme ligand]

[1] Yu L., Wei Y.-Y., Usui S., Yu C.-A. J. Biol. Chem. 267:24508-24515(1992).[2] Abraham P.R., Mulder A., Van't Riet J., Raue H.A. Mol. Gen. Genet. 242:708-716(1994).[3] Leblanc C., Boyen C., Richard O., Bonnard G., Grienenberger J.M., Kloareg B. J. Mol. Biol. 250:484-495(1995).

604. Sec1 family

[1] The Secl family: a novel family of proteins involved in synaptic transmission and general secretion. Halachmi N, Lev Z; J Neurochem 1996;66:889-897.
Number of members: 40

605. Protein secE/sec61-gamma signature

In bacteria, the secE protein plays a role in protein export; it is one of the components - with secY and secA - of the preprotein translocase. In eukaryotes, the evolutionary related protein sec61-gamma playsa role in protein translocation through the endoplasmic reticulum; it is part of a trimeric complex that also consist of sec61-alpha and beta [1]. Both secE and sec61-gamma are small proteins of about 60 to 90 amino acids that contain a single transmembrane region at their C-terminal extremity (Escherichia colisecE is an exception, in that it possess an extra N-terminal segment of 60residues that contains two additional transmembrane domains).The sequence of secE/sec61-gamma is not extremely well conserved, however it is possible to derive a signature pattern centered on a conserved proline located 10 residues before the beginning of the transmembrane domain.
Consensus pattern: [LIVMFY]-x(2)-[DENQGA]-x(4)-[LIVMFTA]-x-[KRV]-x(2)-[KW]-P-x(3)-[SEQ]-x(7)-[LIVT]-[LIVGA]-[LIVFGAST]
[1] Hartmann E., Sommer T., Prehn S., Goerlich D., Jentsch S., Rapoport T.A. Nature 367:654-657(1994).

606. 11-S plant seed storage proteins signature

Plant seed storage proteins, whose principal function appears to be the major nitrogen source for the developing plant, can be classified, on the basis of their structure, into different families. 11-S are non-glycosylated proteins which form hexameric structures [1,2]. Each of the subunits in the hexamer is itself composed of an acidic and a basic chain derived from a single precursor and linked by a disulfide bond. This structure is shown in the following representation. +------------------------+ | | xxxxxxxxxxxCxxxxxxxxxxxxxxxxxxxxxxNGxCxxxxxxxxxxxxxxxxxxxxxxx ******** <-----Acidic-subunit------------><-----Basic-subunit------> <----------------About-480-to-500-residues---------------->'C': conserved cysteine involved in a disulfide bond.'*': position of the pattern. Proteins that belong to the 11-S family are: pea and broad bean legumins, rape cruciferin, rice glutelins, cotton beta-globulins, soybean glycinins, pumpkin 11-S globulin, oat globulin, sunflower helianthinin G3, etc. The region that includes the conserved cleavage site between the acidic and basic subunits (Asn-Gly) and a proximal cysteine residue which is involved in the interchain disulfide bond have been used as a signature pattern for this family of proteins.

Consensus pattern: N-G-x-[DE](2)-x-[LIVMF]-C-[ST]-x(11,12)-[PAG]-D [C is involved in a disulfide bond

[1] Hayashi M., Mori H., Nishimura M., Akazawa T., Hara-Nishimura I. Eur. J. Biochem. 172:627-632(1988).[2] Shotwell M.A., Afonso C., Davies E., Chesnut R.S., Larkins B.A. Plant Physiol. 87:698-704(1988).

607. 7S seed storage protein

7S globulin is one of the main storage proteins of most angiosperms and gymnosperms. The 7S storage proteins are homotrimers.

Number of members: 67

[1] The three-dimensional structure of canavalin from jack bean (Canavalia ensiformis). Ko TP, Ng JD, McPherson A; Plant Physiol 1993;101:729-744.

09595326.051500

608. Aspartate-semialdehyde dehydrogenase signature

Aspartate-semialdehyde dehydrogenase (ASD) catalyzes the second step in the common biosynthetic pathway leading from Asp to diaminopimelate and Lys, to Met, and to Thr; the NADP-dependent reductive dephosphorylation of L-aspartyl phosphate to L-aspartate-semialdehyde. In bacteria and fungi, ASDis a protein of about 40 Kd (340 to 370 residues) whose sequence is not extremely well conserved [1]. A conserved cysteine residue has been implicated as important for the catalytic activity [2].The region of conservation around the active site residue is too small to be used as signature pattern. Another more conserved region, located in the last third of the sequence, and which contains both a conserved cysteine as well as an histidine has been used instead.

Consensus pattern: [LIVM]-[SADN]-x(2)-C-x-R-[LIVM]-x(4)-[GSC]-H-[STA

[1] Baril C., Richaud C., Fourni E., Baranton G., Saint Girons I. J. Gen. Microbiol. 138:47-53(1992).[2] Karsten W.E., Viola R.E. Biochim. Biophys. Acta 1121:234-238(1992).

N-acetyl-gamma-glutamyl-phosphate reductase active site

N-acetyl-gamma-glutamyl-phosphate reductase (EC 1.2.1.38) (AGPR) [1,2] is the enzyme that catalyzes the third step in the biosynthesis of arginine from glutamate, the NADP-dependent reduction of N-acetyl-5-glutamyl phosphate into N-acetylglutamate 5-semialdehyde.In bacteria it is a monofunctional protein of 35 to 38 Kd (gene argC) while in fungi it is part of a bifunctional mitochondrial enzyme (gene ARG5,6, arg11 orarg-6) which contains a N-terminal acetylglutamate kinase (EC 2.7.2.8) domain and a C-terminal AGPR domain. In the Escherichia coli enzyme, a cysteine has been shown to be implicated in the catalytic activity, the region around this residue is well conserved and can be used as a signature pattern.

Consensus pattern: [LIVM]-[GSA]-x-P-G-C-[FY]-[AVP]-T-[GA]-x(3)-[GTAC]-[LIVM]- x-P [C is the active site residue]

[1] Ludovice M., Martin J.F., Carrachas P., Liras P. J. Bacteriol. 174:4606-4613(1992).[2] Gessert S.F., Kim J.H., Nargang F.E., Weiss R.L. J. Biol. Chem. 269:8189-8203(1994).

09595565.061800

609. Sialyltransferase family,

Number of members: 18

610. SpoU rRNA Methylase family

This family of proteins probably use S-AdoMet. Number of members: 58

[1] SpoU protein of Escherichia coli belongs to a new family of putative rRNA methylases. Koonin EV, Rudd KE; Nucleic Acids Res 1993;21:5519-5519. [2] The spoU gene of escherichia coli , the fourth gene of the spoT operon, is essential for tRNA (Gm18) 2 ' methyltransferase activity. Persson BC, Jager G, Gustafsson C; Nucleic Acids Res 1997;25:4093-4097.

611. Stathmin family signatures

Stathmin [1] (from the Greek 'stathmos' which means relay), is an ubiquitous intracellular protein, present in a variety of phosphorylated forms and which serves as a relay for diverse

second messenger pathways. Its expression and phosphorylation are regulated throughout development and in response to extracellular signals regulating cell proliferation, differentiation and function. Stathmin is a highly conserved protein of 149 amino acid residues. Structurally, it consists of an N-terminal domain of about 45 residues followed by a 78 residue alpha-helical domain consisting of a heptad repeat coiled coil structure and a C-terminal domain of 25 residues. Protein SCG10 is a neuron-specific, membrane-associated protein that accumulates in the growth cones of developing neurons. It is highly similar in its sequence to stathmin, but differs in that it contains an additional N-terminal hydrophobic segment of 32 residues which is probably responsible for its interaction with membranes. Xenopus protein XB3 is also evolutionary related to stathmin and also contains an additional N-terminal hydrophobic domain [2]. A conserved decapeptide which ends with the first three residues of the coiled coil domain and a second pattern that corresponds to part of the central region of the coiled coil have been selected as signatures for proteins of the stathmin family.
Consensus pattern: P-[KRQ]-[KR](2)-[DE]-x-S-L-[EG]-E-
Consensus pattern: A-E-K-R-E-H-E-[KR]-E-
[1] Sobel A. Trends Biochem. Sci. 16:301-305(1991).[2] Maucuer A., Moreau J., Mechali M., Sobel A. J. Biol. Chem. 268:16420-16429(1993).

09555295 . 051502

612. SUA5/yciO/yrdC family signature. The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast protein SUA5. - Escherichia coli hypothetical protein yciO and HI1198, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yrdC and HI0656, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein ywlC. - Mycobacterium leprae hypothetical protein in rfe-hemK intergenic region. - Methanococcus jannaschii hypothetical protein MJ0062.These are proteins of from 20 to 46 Kd which contain a number of conserved regions in their N-terminal section. They can be picked up in the database by the following pattern.

Consensus pattern: [LIVMTA](3)-[LIVMFYC]-[PG]-T-[DE]-[STA]-x-[FY]-[GA]- [LIVM]-[GS]-

[1] Bairoch A., Rudd K.E., Robison K. Unpublished observations (1995).

613. Sucrose synthase

Sucrose synthases catalyse the synthesis of sucrose from UDP-glucose and fructose. This family includes the bulk of the sucrose synthase protein. However the carboxyl terminal region of the sucrose synthases belongs to the glycosyl transferase family Glycos_transf_1.

614. Sulfotransferase proteins

Number of members: 59

09595673 .072502

615. Synaptophysin / synaptoporin signature

Synaptophysin and synaptoporin [1] are structurally related proteins, found in the membrane of synaptic vesicles, which may function as ionic or solute channels. These two glycoproteins seem to span the membrane four times. Both their N- and C-termini sequences seem to be cytoplasmically located. As a signature pattern for this family of proteins, a highly conserved region located in the beginning of the first intravesicular loop just after the first transmembrane domain has been selected. This region contains a cysteine residue that may be involved in a disulfide bond.

Consensus pattern: L-S-V-[DE]-C-x-N-K-T [C may be involved in a disulfide bond

[1] Knaus P., Marqueze-Pouey B., Scherer H., Betz H. Neuron 5:453-462(1990).

616. Syndecans signature

Syndecans [1,2] (from the greek syndein; to bind together) are a family of transmembrane heparan sulfate proteoglycans which are implicated in the binding of extracellular matrix components and growth factors. Syndecans bind a variety of molecules via their heparan sulfate chains and can act as receptors or as co-receptors. Structurally, these proteins consist of four separate domains: a) A signal sequence; b) An extracellular domain (ectodomain) of variable length and whose sequence is not evolutionary conserved in the various forms of syndecans. The ectodomain contains the sites of attachment of the heparan sulfate glycosaminoglycan side chains; c) A transmembrane region; d) A highly conserved cytoplasmic domain of about 30 to 35 residues which could interact with cytoskeletal proteins. The proteins known to belong to this family are: - Syndecan 1. - Syndecan 2 or fibroglycan. - Syndecan 3 or neuroglycan or N-syndecan. - Syndecan 4 or amphiglycan or ryudocan. - Drosophila syndecan. - Caenorhabditis elegans probable syndecan (F57C7.3).The signature pattern that has been developed for syndecans starts with the last residue of the transmembrane region and includes the first 10 residues of the cytoplasmic domain. This region, which contains four basic residues, could act as a stop transfer site.

Consensus pattern: [FY]-R-[IM]-[KR]-K(2)-D-E-G-S-Y

[1] Bernfield M., Kokenyesi R., Kato M., Hinkes M.T., Spring J., Gallo R.L., Lose E.J. Annu. Rev. Cell Biol. 8:365-393(1992).[2] David G. FASEB J. 7:1023-1030(1993).

617. Syntaxin / epimorphin family signature

The following proteins have been shown to be evolutionary related [1,2,3]: - Epimorphin (or syntaxin 2), a mammalian mesenchymal protein which plays an essential role in epithelial morphogenesis. - Syntaxin 1A (also known as antigen HPC-1) and syntaxin 1B which are synaptic proteins which may be involved in docking of synaptic vesicles at presynaptic active zones. - Syntaxin 3. - Syntaxin 4, which is potentially involved in docking of synaptic vesicles at presynaptic active zones. - Syntaxin 5, which mediates endoplasmic reticulum to golgi transport. - Syntaxin 6, which is involved in intracellular vesicle trafficking. - Syntaxin 7. - Yeast PEP12 (or VPS6) which is required for the transport of proteases to the vacuole. - Yeast SED5 which is required for the fusion of transport vesicles with the Golgi complex. - Yeast SSO1 and SSO2 which are required for vesicle fusion with the plasma membrane. - Yeast VAM3, which is required for vacuolar assembly. - Arabidopsis thaliana protein KNOLLE which may be involved in cytokinesis. - Caenorhabditis elegans hypothetical proteins F35C8.4, F48F7.2, F55A11.2 and T01B11.3.The above proteins share the following characteristics: a size ranging from30 Kd to 40 Kd; a C-terminal extremity which is highly hydrophobic and isprobably involved in anchoring the protein to the membrane; a central, well conserved region, which seems to be in a coiled-coil conformation. The pattern specific for this family is based on the most conserved region of the coiled coil domain.

Consensus pattern: [RQ]-x(3)-[LIVMA]-x(2)-[LIVM]-[ESH]-x(2)-[LIVMT]-x-[DEVM]-[LIVM]-x(2)-[LIVM]-[FS]-x(2)-[LIVM]-x(3)-[LIVT]-x(2)-Q- [GADEQ]-x(2)-[LIVM]-[DNQT]-x-[LIVMF]-[DESV]-x(2)-[LIVM]

[1] Bennett M.K., Garcia-Arraras J.E., Elferink L.A., Peterson K., Fleming A.M., Hazuka C.D., Scheller R.H. Cell 74:863-873(1993).[2] Spring J., Kato M., Bernfield M. Trends Biochem. Sci. 18:124-125(1993).[3] Pelham H.R.B. Cell 73:425-426(1993).

618. Sm protein

The U1, U2, U4/U6, and U5 small nuclear ribonucleoprotein particles (snRNPs) involved in pre-mRNA splicing contain seven Sm proteins (B/B', D1, D2, D3, E, F and G) in common, which assemble around the Sm site present in four of the major spliceosomal small nuclear RNAs. These proteins contain a common sequence motif in two segments, Sm1 and Sm2, separated by a short variable linker.

[1] Hermann H, Fabrizio P, Raker VA, Foulaki K, Hornig H, Brahms H, Luhrmann R EMBO J 1995;14:2076-2088. [2] Kambach C, Walke S, Young R, Avis JM, de la Fortelle E, Raker VA, Luhrmann R, Li J, Nagai K; Cell 1999;96:375-387.

619. Skp1 family

[1] Stebbins CE, Kaelin WG Jr, Pavletich NP; Science 1999;284:455-461.

620. Protein secY signatures

The eubacterial secY protein [1] plays an important role in protein export. It interacts with the signal sequences of secretory proteins as well as with two other components of the protein translocation system: secA and secE. SecY is an integral plasma membrane protein of 419 to 492 amino acid residues that apparently contains ten transmembrane segments. Such a structure probablyconfers to secY a 'translocator' function, providing a channel for periplasmic and outer-membrane precursor proteins.Homologs of secY are found in archaebacteria [2]. SecY is also encoded in the chloroplast genome of some algae [3] where it could be involved in a prokaryotic-like protein export system across the two membranes of the chloroplast endoplasmic reticulum (CER) which is present in chromophyte andcryptophyte algae. Two signature patterns have been developed for secY proteins. The first corresponds to the second transmembrane region, which is the most conserved section of these proteins. The second spans the C-terminal part of the fourth transmembrane region, a short intracellular loop, and the N-terminal part of the fifth transmembrane region.

Consensus pattern: [GST]-[LIVMF](2)-x-[LIVM]-G-[LIVM]-x-P-[LIVMFY](2)-x-[AS]-[GSTQ]-[LIVMFAT](3)-Q-[LIVMFA](2)

Consensus pattern: [LIVMFYW](2)-x-[DE]-x-[LIVMF]-[STN]-x(2)-G-[LIVMF]-[GST]-[NST]-G-x-[GST]-[LIVMF](3)

[1] Ito K. Mol. Microbiol. 6:2423-2428(1992).[2] Auer J., Spicker G., Boeck A. Biochimie 73:683-688(1991).[3] Douglas S.E. FEBS Lett. 298:93-96(1992).

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621. (Seed protein) Small hydrophilic plant seed proteins signature. The following small hydrophilic plant seed proteins are structurally related: - Arabidopsis thaliana proteins GEA1 and GEA6. - Cotton late embryogenesis abundant (LEA) protein D-19. - Carrot EMB-1 protein. - Barley LEA proteins B19.1A, B19.1B, B19.3 and B19.4. - Maize late embryogenesis abundant protein Emb564. - Radish late seed maturation protein p8B6. - Rice embryonic abundant protein Emp1. - Sunflower 10 Kd late embryogenesis abundant protein (DS10). - Wheat Em proteins. These proteins contains from 83 to 153 amino acid residues and may play a role[1,2] in equipping the seed for survival, maintaining a minimal level of hydration in the dry organism and preventing the denaturation of cytoplasmic components. They may also play a role during imbibition by controlling water uptake. As a signature pattern, the best conserved region in the sequence of these proteins has been developed, it is a glycine-rich nonapeptide located in the N-terminal section.-

Consensus pattern: G-[EQ]-T-V-V-P-G-G-T-

[1] Dure L. III, Crouch M., Harada J., Ho T.-H. D., Mundy J., Quatrano R., Thomas T., Sung Z.R. Plant Mol. Biol. 12:475-486(1989).[2] Gaubier P., Raynal M., Hull G., Huestis G.M., Grellet F., Arenas C., Pages M., Delseny M. Mol. Gen. Genet. 238:409-418(1993).

622. Serine carboxypeptidases, active sites

All known carboxypeptidases are either metallo carboxypeptidases or serinecarboxypeptidases. The catalytic activity of the serine carboxypeptidases, like that of the trypsin family serine proteases, is provided by a charge relay system involving an aspartic acid residue hydrogen-bonded to a histidine, which is itself hydrogen-bonded to a serine [1]. Proteins known to be serine carboxypeptidases are: - Barley and wheat serine carboxypeptidases I, II, and III [2]. - Yeast carboxypeptidase Y (YSCY) (gene PRC1), a vacuolar protease involved in degrading small peptides. - Yeast KEX1 protease, involved in killer toxin and alpha-factor precursor processing. - Fission yeast sxa2, a probable carboxypeptidase involved in degrading or processing mating pheromones [3]. - Penicillium janthinellum carboxypeptidase S1 [4]. - Aspergullus niger carboxypeptidase pepF. - Aspergullus satoi carboxypeptidase cpdS. - Vertebrate protective protein / cathepsin A [5], a lysosomal protein which is not only a carboxypeptidase but also essential for the activity of both beta-galactosidase and neuraminidase. - Mosquito vitellogenic carboxypeptidase (VCP) [6]. - Naegleria fowleri virulence-related protein Nf314 [7]. - Yeast hypothetical protein YBR139w. - Caenorhabditis elegans hypothetical proteins C08H9.1, F13D12.6, F32A5.3, F41C3.5 and K10B2.2.This family also includes: - Sorghum (s)-hydroxymandelonitrile lyase (hydroxynitrile lyase) (HNL) [8], an enzyme involved in plant cyanogenesis. The sequences surrounding the active site serine and histidine residues are highly conserved in all these serine carboxypeptidases.

Consensus pattern: [LIVM]-x-[GTA]-E-S-Y-[AG]-[GS] [S is the active site residue]

Consensus pattern: [LIVF]-x(2)-[LIVSTA]-x-[IVPST]-x-[GSDNQL]-[SAGV]-[SG]-H-x-[IVAQ]-P-x(3)-[PSA] [H is the active site residue]

[1] Liao D.I., Remington S.J. J. Biol. Chem. 265:6528-6531(1990).[2] Sorensen S.B., Svendsen I., Breddam K. Carlsberg Res. Commun. 54:193-202(1989).[3] Imai Y., Yamamoto M. Mol. Cell. Biol. 12:1827-1834(1992).[4] Svendsen I., Hofmann T., Endrizzi J., Remington J., Breddam K. FEBS Lett. 333:39-43(1993).[5] Galjart N.J., Morreau H., Willemsen R., Gillemans N., Bonten E.J., d'Azzo A. J. Biol. Chem. 266:14754-14762(1991).[6] Cho W.L., Deitsch K.W., Raikhel A.S. Proc. Natl. Acad. Sci. U.S.A. 88:10821-10824(1991).[7] Hu W.N., Kopachik W., Band R.N. Infect. Immun. 60:2418-2424(1992).[8] Wajant H., Mundry K.W., Pfitzenmaier K. Plant Mol. Biol. 26:735-746(1994).[9] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]

623. Serpins signature. Serpins (SERine Proteinase INhibitors) [1,2,3,4] are a group of structurally related proteins. They are high molecular weight (400 to 500 amino acids),extracellular, irreversible serine protease inhibitors with a well defined structural-functional characteristic: a reactive region that acts as a 'bait' for an appropriate serine protease. This region is found in the C-terminal part of these proteins. Proteins which are known to belong to the serpin family are listed below (references are only provided for recently determined sequences): - Alpha-1 protease inhibitor (alpha-1-antitrypsin, contrapsin). - Alpha-1-antichymotrypsin, - Antithrombin III. - Alpha-2-antiplasmin. - Heparin cofactor II. - Complement C1 inhibitor. - Plasminogen activator inhibitors 1 (PAI-1) and 2 (PAI-2). - Glia derived nexin (GDN) (Protease nexin I). - Protein C inhibitor. - Rat hepatocytes SPI-1, SPI-2 and SPI-3 inhibitors. - Human squamous cell carcinoma antigen (SCCA) which may act in the modulation of the host immune response against tumor cells. - A lepidopteran protease inhibitor. - Leukocyte elastase inhibitor which, in contrast to other serpins, is an intracellular protein. - Neuroserpin [5], a neuronal inhibitor of plasminogen activators and plasmin. - Cowpox virus crmA [6], an inhibitor of the thiol protease interleukin-1B converting enzyme (ICE). CrmA is the only serpin known to inhibit a non-serine proteinase. - Some orthopoxviruses probable protease inhibitors, which may be involved in the regulation of the blood clotting cascade and/or of the complement cascade in the mammalian host. On the basis of strong sequence similarities, a number of proteins with no known inhibitory activity are said to belong to this family: - Birds ovalbumin and the related genes X and Y proteins. - Angiotensinogen; the precursor of the angiotensin active peptide. - Barley protein Z; the major endosperm albumin. - Corticosteroid binding globulin (CBG). - Thyroxine-binding globulin (TBG). - Sheep uterine milk protein (UTMP) and pig uteroferrin-associated protein (UFAP). - Hsp47, an endoplasmic reticulum heat-shock protein that binds strongly to collagen and could act as a chaperone in the collagen biosynthetic pathway [7]. - Maspin, which seems to function as a tumor supressor [5]. - Pigment epithelium-derived factor precursor (PEDF), a protein with a strong neutrophic activity [8]. - Ep45, an estrogen-regulated protein from Xenopus [9]. A signature pattern has been developed for this family of proteins, centered on a well conserved Pro-Phe sequence which is found ten to fifteen residues on the C-terminal side of the reactive bond

Consensus pattern: [LIVMFY]-x-[LIVMFYAC]-[DNQ]-[RKHQS]-[PST]-F-[LIVMFY]-[LIVMFYC]-x-[LIVMFAH]-

[1] Carrell R., Travis J. Trends Biochem. Sci. 10:20-24(1985).[2] Carrell R., Pemberton P.A., Boswell D.R. Cold Spring Harbor Symp. Quant. Biol. 52:527-535(1987).[3] Huber R., Carrell R.W. Biochemistry 28:8951-8966(1989).[4] Remold-O'Donneel E. FEBS Lett. 315:105-108(1993).[5] Osterwalder T., Contartese J., Stoeckli E.T., Kuhn T.B., Sonderegger P. EMBO J. 15:2944-2953(1996).[6] Komiyama T., Ray C.A., Pickup D.J., Howard A.D., Thornberry N.A., Peterson E.P., Salvesen G. J. Biol. Chem. 269:19331-19337(1994).[7] Clarke E., Sandwal B.D. Biochim. Biophys. Acta 1129:246-248(1992).[8] Zou Z., Anisowicz A., Neveu M., Rafidi K., Sheng S., Sager R., Hendrix M.J., Seftor E., Thor A. Science 263:526-529(1994).[9] Steele F.R., Chader G.J., Johnson L.V., Tombran-Tink J. Proc. Natl. Acad. Sci. U.S.A. 90:1526-1530(1993).[10] Holland L.J., Suksang C., Wall A.A., Roberts L.R., Moser D.R., Bhattacharya A. J. Biol. Chem. 267:7053-7059(1992).

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624. Sigma-54 interaction domain signatures and profile

Some bacterial regulatory proteins activate the expression of genes from promoters recognized by core RNA polymerase associated with the alternative sigma-54 factor. These have a conserved domain of about 230 residues involved in the ATP-dependent [1,2] interaction with sigma-54. This domain has been found in the proteins listed below: - acoR from Alcaligenes eutrophus, an activator of the acetoin catabolism operon acoXABC. - algB from Pseudomonas aeruginosa, an activator of alginate biosynthetic gene algD. - dctD from Rhizobium, an activator of dctA, the C4-dicarboxylate transport protein. - dhaR from Citrobacter freundii, a regulator of the dha operon for glycerol utilization. - fhlA from Escherichia coli, an activator of the formate dehydrogenase H and hydrogenase III structural genes. - flbD from Caulobacter crescentus, an activator of flagellar genes. - hoxA from Alcaligenes eutrophus, an activator of the hydrogenase operon. - hrpS from Pseudomonas syringae, an activator of hprD as well as other hrp loci involved in plant pathogenicity. - hupR1 from Rhodobacter capsulatus, an activator of the [NiFe] hydrogenase genes hupSL. - hydG from Escherichia coli and Salmonella typhimurium, an activator of the hydrogenase activity. - levR from Bacillus subtilis, which regulates the expression of the levanase operon (levDEFG and sacC). - nifA (as well as anfA and vnfA) from various bacteria, an activator of the nif nitrogen-fixing operon. - ntrC, from various bacteria, an activator of nitrogen assimilatory genes such as that for glutamine synthetase (glnA) or of the nif operon. - pgtA from Salmonella typhimurium, the activator of the inducible phospho- glycerate transport system. - pilR from Pseudomonas aeruginosa, an activator of pilin gene transcription. - rocR from Bacillus subtilis, an activator of genes for arginine utilization - tyrR from Escherichia coli, involved in the transcriptional regulation of aromatic amino-acid biosynthesis and transport. - wtsA, from Erwinia stewartii, an activator of plant pathogenicity gene wtsB. - xylR from Pseudomonas putida, the activator of the tol plasmid xylene catabolism operon xylCAB and of xylS. - Escherichia coli hypothetical protein yfhA. - Escherichia coli hypothetical protein yhgB. About half of these proteins (algB, dcdT, flbD, hoxA, hupR1, hydG, ntrC, pgtA and pilR) belong to signal transduction two-component systems [3] and possess a domain that can be phosphorylated by a sensor-kinase protein in their N- terminal section. Almost all of these proteins possess a helix-turn-helix DNA-binding domain in their C-terminal section. The domain which interacts with the sigma-54 factor has an ATPase activity. This may be required to promote a conformational change necessary for theinteraction [4]. The domain contains an atypical ATP-binding motif A (P-loop) as well as a form of motif B. The two ATP-binding motifs are located in the N-terminal section of the domain; signature patterns have been developed for both motifs. Other regions of the domain are also conserved. One of them, located in the C-terminal section, has been selected as a third signature pattern.

Consensus pattern: [LIVMFY](3)-x-G-[DEQ]-[STE]-G-[STAV]-G-K-x(2)-[LIVMFY]

Consensus pattern: [GS]-x-[LIVMF]-x(2)-A-[DNEQASH]-[GNEK]-G-[STIM]-[LIVMFY](3)-[DE]-[EK]-[LIVM]

Consensus pattern: [FYW]-P-[GS]-N-[LIVM]-R-[EQ]-L-x-[NHAT]

[1] Morrett E., Segovia L. J. Bacteriol. 175:6067-6074(1993).[2] Austin S., Kundrot C., Dixon R. Nucleic Acids Res. 19:2281-2287(1991).[3] Albright L.M., Huala E., Ausubel F.M. Annu. Rev. Genet. 23:311-336(1989).[4] Austin S., Dixon R. EMBO J. 11:2219-2228(1992).

625. Sigma-70 factors family signatures

Sigma factors [1] are bacterial transcription initiation factors that promote the attachment of the core RNA polymerase to specific initiation sites and arethen released. They alter the specificity of promoter recognition. Most bacteria express a multiplicity of sigma factors. Two of these factors, sigma-70 (gene rpoD), generally known as the major or primary sigma factor, and sigma-54 (gene rpoN or ntrA) direct the transcription of a wide variety of genes. The other sigma factors, known as alternative sigma factors, are required for the transcription of specific subsets of genes. With regard to sequence similarity, sigma factors can be grouped into two classes: the sigma-54 and sigma-70 families. The sigma-70 family includes, in addition to the primary sigma factor, a wide variety of sigma factors, some of which are listed below: - Bacillus sigma factors involved in the control of sporulation-specific genes: sigma-E (sigE or spoIIGB), sigma-F (sigF or spoIIAC), sigma-G (sigG or spoIIIG), sigma-H (sigH or spo0C) and sigma-K (sigK or spoIVCB/spoIIIC). - Escherichia coli and related bacteria sigma-32 (gene rpoH or htpR) involved in the expression of heat shock genes. - Escherichia coli and related bacteria sigma-27 (gene fliA) involved in the expression of the flagellin gene. - Escherichia coli sigma-S (gene rpoS or katF) which seems to be involved in the expression of genes required for protection against external stresses. - Myxococcus xanthus sigma-B (sigB) which is essential for the late-stage differentiation of that bacteria. Alignments of the sigma-70 family permit the identification of four regions of high conservation [2,3]. Each of these four regions can in turn be subdivided into a number of sub-regions. Signature patterns based on the two best-conserved sub-regions have been developed. The first pattern corresponds to sub-region 2.2;the exact function of this sub-region is not known although it could be involved in the binding of the sigma factor to the core RNA polymerase. The second pattern corresponds to sub-region 4.2 which seems to harbor a DNA-binding 'helix-turn-helix' motif involved in binding the conserved -35region of promoters recognized by the major sigma factors. The second pattern starts one residue before the N-terminal extremity of the HTH region and ends six residues after its C-terminal extremity.

Consensus pattern: [DE]-[LIVMF](2)-[HEQS]-x-G-x-[LIVMFA]-G-L-[LIVMFYE]-x-[GSAM]-[LIVMAP]

Consensus pattern: [STN]-x(2)-[DEQ]-[LIVM]-[GAS]-x(4)-[LIVMF]-[PSTG]-x(3)-[LIVMA]-x-[NQR]-[LIVMA]-[EQH]-x(3)-[LIVMFW]-x(2)-[LIVM]

[1] Helmann J.D., Chamberlin M.J. Annu. Rev. Biochem. 57:839-872(1988).[2] Gribskov M., Burgess R.R. Nucleic Acids Res. 14:6745-6763(1986).[3] Lonetto M.A., Gribskov M., Gross C.A. J. Bacteriol. 174:3843-3849(1992).[4] Lonetto M.A., Brown K.L., Rudd K.E., Buttner M.J. Proc. Natl. Acad. Sci. U.S.A. 91:7573-7577(1994).

626. Signal carboxyl-terminal domain. 430 members.

627. Signal peptidases I signatures

Signal peptidases (SPases) [1] (also known as leader peptidases) remove the signal peptides from secretory proteins. In prokaryotes three types of Spases are known: type I (gene lepB) which is responsible for the processing of the majority of exported pre-proteins; type II (gene lsp) which only process lipoproteins, and a third type involved in the processing of pili subunits. SPase I is an integral membrane protein that is anchored in the cytoplasmic membrane by one (in B. subtilis) or two (in E. coli) N-terminal transmembrane domains with the main part of the protein protuding in the periplasmic space. Two residues have been shown [2,3] to be essential for the catalytic activity of SPase I: a serine and an lysine.SPase I is evolutionary related to the yeast mitochondrial inner membrane protease subunit 1 and 2 (genes IMP1 and IMP2) which catalyze the removal of signal peptides required for the targeting of proteins from the mitochondrial matrix, across the inner membrane, into the inter-membrane space [4]. In eukaryotes the removal of signal peptides is effected by an oligomeric enzymatic complex composed of at least five subunits: the signal peptidase complex (SPC). The SPC is located in the endoplasmic reticulum membrane. Two components of mammalian SPC, the 18 Kd (SPC18) and the 21 Kd (SPC21) subunits as well as the yeast SEC11 subunit have been shown [5] to share regions of sequence similarity with prokaryotic SPases I and yeast IMP1/IMP2. Three signature patterns for these proteins have been developed. The first signature contains the putative active site serine, the second signature contains the putative active site lysine which is not conserved in the SPC subunits, and the third signature corresponds to a conserved region of unknown iological significance which is located in the C-terminal section of all these proteins.

Consensus pattern: [GS]-x-S-M-x-[PS]-[AT]-[LF] [S is an active site residue]

Consensus pattern: K-R-[LIVMSTA](2)-G-x-[PG]-G-[DE]-x-[LIVM]-x-[LIVMFY] [K is an active site residue]

Consensus pattern: [LIVMFYW](2)-x(2)-G-D-[NH]-x(3)-[SND]-x(2)-[SG]

[1] Dalbey R.E., von Heijne G. Trends Biochem. Sci. 17:474-478(1992).[2] Sung M., Dalbey R.E. J. Biol. Chem. 267:13154-13159(1992).[3] Black M.T. J. Bacteriol. 175:4957-4961(1993).[4] Nunnari J., Fox T.D., Walter P. Science 262:1997-2004(1993).[5] van Dijl

J.M., de Jong A., Vehmaanpera J., Venema G., Bron S. EMBO J. 11:2819-2828(1992).[6] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]

628. (sodcu) Copper/Zinc superoxide dismutase signatures

Copper/Zinc superoxide dismutase (SODC) [1] is one of the three forms of an enzyme that catalyzes the dismutation of superoxide radicals. SODC binds one atom each of zinc and copper. Various forms of SODC are known: acytoplasmic form in eukaryotes, an additional chloroplast form in plants, an extracellular form in some eukaryotes, and a periplasmic form in prokaryotes. The metal binding sites are conserved in all the known SODC sequences [2]. Two signature patterns have been derived for this family of enzymes: the first one contains two histidine residues that bind the copper atom; the second one islocated in the C-terminal section of SODC and contains a cysteine which is involved in a disulfide bond.

Consensus pattern: [GA]-[IMFAT]-H-[LIVF]-H-x(2)-[GP]-[SDG]-x-[STAGDE] [The two H's are copper ligands]

Consensus pattern: G-[GN]-[SGA]-G-x-R-x-[SGA]-C-x(2)-[IV] [C is involved in a disulfide bond]

[1] Bannister J.V., Bannister W.H., Rotilio G. CRC Crit. Rev. Biochem. 22:111-154(1987).[2] Smith M.W., Doolittle R.F. J. Mol. Evol. 34:175-184(1992).

629. (sodfe) Manganese and iron superoxide dismutases signature

Manganese superoxide dismutase (SODM) [1] is one of the three forms of an enzyme that catalyzes the dismutation of superoxide radicals. The four ligands of the manganese atom are conserved in all the known SODM sequences. These metal ligands are also conserved in the related iron form ofsuperoxide dismutases [2,3]. A short conserved region which includes two of the four ligands: an aspartate and a histidine has been selected as a signature.

Consensus pattern: D-x-W-E-H-[STA]-[FY](2) [D and H are manganese/iron ligands]

[1] Bannister J.V., Bannister W.H., Rotilio G. CRC Crit. Rev. Biochem. 22:111-154(1987).[2] Parker M.W., Blake C.C.F. FEBS Lett. 229:377-382(1988).[3] Smith M.W., Doolittle R.F. J. Mol. Evol. 34:175-184(1992).

630. Spectrin repeat

Spectrin repeats are found in several proteins involved in cytoskeletal structure. These include spectrin, alpha-actinin and dystrophin.The sequence repeat used in this family is taken from the structural repeat in reference [2]. The spectrin repeat forms a three helix bundle. The second helix is interrupted by proline in some sequences.

Number of members: 898

[1] Actin-binding proteins. 1: Spectrin super family. Hartwig JH; Protein Profile 1995;2:732-732. [2] Crystal structure of the repetitive segments of spectrin. Yan Y, Winograd E, Viel A, Cronin T, Harrison SC, Branton D; Science 1993;262:2027-2030.

631. (subtilase) Streptomyces subtilisin-type inhibitors signature

Bacteria of the Streptomyces family produce a family of proteinase inhibitors[1] characterized by their strong activity toward subtilisin. They arecollectively known as SSI's: Streptomyces Subtilisin Inhibitors. Some SSI'salso inhibit trypsin or chymotrypsin. In their mature secreted form, SSI's areproteins of about 110 residues with two conserved disulfide bonds. +-------+ +--------------+ | | | | xxxxxxxxxxxxxxxCxxxxxxxCxxxxxxxxxxCx#xxxxxxxxxxxxxCxxxxxxx ************'C': conserved cysteine involved in a disulfide bond.'#': active site residue.'*': position of the pattern.

Consensus pattern: C-x-P-x(2,3)-G-x-H-P-x(4)-A-C-[ATD]-x-L [The two C's are involved in a disulfide bond]

[1] Taguchi S., Kojima S., Terabe M., Miura K.-I., Momose H. Eur. J. Biochem. 220:911-918(1994).

632. Sugar transport proteins signatures

In mammalian cells the uptake of glucose is mediated by a family of closely related transport proteins which are called the glucose transporters [1,2,3].At least seven of these transporters are currently known to exist (in Human they are encoded by the GLUT1 to GLUT7 genes).These integral membrane proteins are predicted to comprise twelve membrane spanning domains. The glucose transporters show sequence similarities [4,5] with a number of other sugar or metabolite transport proteins listed below (references are only provided for recently determined sequences). - Escherichia coli arabinose-proton symport (araE). - Escherichia coli galactose-proton symport (galP). - Escherichia coli and Klebsiella pneumoniae citrate-proton symport (also known as citrate utilization determinant) (gene cit). - Escherichia coli alpha-ketoglutarate permease (gene kgtP). - Escherichia coli proline/betaine transporter (gene proP) [6]. - Escherichia coli xylose-proton symport (xylE). - Zymomonas mobilis glucose facilitated diffusion protein (gene glf). - Yeast high and low affinity glucose transport proteins (genes SNF3, HXT1 to HXT14). - Yeast galactose transporter (gene GAL2). - Yeast maltose permeases (genes MAL3T and MAL6T). - Yeast myo-inositol transporters (genes ITR1 and ITR2). - Yeast carboxylic acid transporter protein homolog JEN1. - Yeast inorganic phosphate transporter (gene PHO84). - Kluyveromyces lactis lactose permease (gene LAC12). - Neurospora crassa quinate transporter (gene Qa-y), and Emericella nidulans quinate permease (gene qutD). - Chlorella hexose carrier (gene HUP1). - Arabidopsis thaliana glucose transporter (gene STP1). - Spinach sucrose transporter. - Leishmania donovani transporters D1 and D2. - Leishmania enriettii probable transport protein (LTP). - Yeast hypothetical proteins YBR241c, YCR98c and YFL040w. - Caenorhabditis elegans hypothetical protein ZK637.1. - Escherichia coli hypothetical proteins yabE, ydjE and yhjE. - Haemophilus influenzae hypothetical proteins HI0281 and HI0418. - Bacillus subtilis hypothetical proteins yxbC and yxdF. It has been suggested [4] that these transport proteins have evolved from theduplication of an ancestral protein with six transmembrane regions, this hypothesis is based on the conservation of two G-R-[KR] motifs. The first one is located between the second and third transmembrane domains and the second one between transmembrane domains 8 and 9. Two patterns have been developed to detect this family of proteins. The first pattern is based on the G-R-[KR] motif; but because this motif is too short to be specific to this family of proteins, a pattern from a larger region centered on the second copy of this motif was derived. The second pattern is based on a number of conserved residues which are located at the end of the fourth transmembrane segment and in the short loop region between the fourth and fifth segments.

Consensus pattern: [LIVMSTAG]-[LIVMFSAG]-x(2)-[LIVMSA]-[DE]-x-[LIVMFYWA]-G- R-[RK]-x(4,6)-[GSTA]

Consensus pattern: [LIVMF]-x-G-[LIVMFA]-x(2)-G-x(8)-[LIFY]-x(2)-[EQ]-x(6)- [RK]

[1] Silverman M. Annu. Rev. Biochem. 60:757-794(1991).[2] Gould G.W., Bell G.I. Trends Biochem. Sci. 15:18-23(1990).[3] Baldwin S.A. Biochim. Biophys. Acta 1154:17-49(1993).[

4] Maiden M.C.J., Davis E.O., Baldwin S.A., Moore D.C.M., Henderson P.J.F. Nature 325:641-643(1987).[5] Henderson P.J.F. Curr. Opin. Struct. Biol. 1:590-601(1991).[6] Culham D.E., Lasby B., Marangoni A.G., Milner J.L., Steer B.A., van Nues R.W., Wood J.M. J. Mol. Biol. 229:268-276(1993).

633. Synaptobrevin signature

Synaptobrevin [1] is an intrinsic membrane protein of small synaptic vesicles whose function is not yet known, but which is highly conserved in mammals, electric ray (where its is known as VAMP-1), Drosophila and yeast [2]. In yeast there are two closely related forms of synaptobrevin (genes SNC1 andSNC2) while in mammals there is at least 4 (genes SYB1, SYB2, SYB3 and SYBL1).Structurally synaptobrevin consist of a N-terminal cytoplasmic domain of from 90 to 110 residues, followed by a transmembrane region, and then by a short (from 2 to 22 residues) C-terminal intravesicular domain. As a signature pattern for synaptobrevin, a highly conserved stretch of residues located in the central part of the sequence was selected.

Consensus pattern: N-[LIVM]-[DENS]-[KL]-V-x-[DEQ]-R-x(2)-[KR]-[LIVM]-[STDE]- x-[LIVM]-x-[DE]-[KR]-[TA]-[DE]

[1] Suedhof T.C., Baumert M., Perin M.S., Jahn R. Neuron 2:1475-1481(1989).[2] Gerst J.E., Rodgers L., Riggs M., Wigler M. Proc. Natl. Acad. Sci. U.S.A. 89:4338-4342(1992).

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634. TBC domain. Identification of a TBC domain in GYP6_YEAST and GYP7_YEAST, which are GTPase activator proteins of yeast Ypt6 and Ypt7, imply that these domains are GTPase activator proteins of Rab-like small GTPases. Number of members: 55

[1] Medline: 96032578. Molecular cloning of a cDNA with a novel domain present in the tre-2 oncogene and the yeast cell cycle regulators BUB2 and cdc16. Richardson PM, Zon LI; Oncogene 1995;11:1139-1148.

[2]Medline: 97398935. A shared domain between a spindle assembly checkpoint protein and Ypt/Rab-specific GTPase-activators. Neuwald AF; Trends Biochem Sci 1997;22:243-244.

635. Transcription factor TFIID repeat signature (TBP)

Transcription factor TFIID (or TATA-binding protein, TBP) [1,2] is a general factor that plays a major role in the activation of eukaryotic genes transcribed by RNA polymerase II. TFIID binds specifically to the TATA box promoter element which lies close to the position of transcription initiation. There is a remarkable degree of sequence conservation of a C-terminal domain of about 180 residues in TFIID from various eukaryotic sources. This region isnecessary and sufficient for TATA box binding. The most significant structural feature of this domain is the presence of two conserved repeats of a 77 amino-acid region. The intramolecular symmetry generates a saddle-shaped structure that sits astride the DNA [3]. Drosophila TRF (TBP-related factor) [4] is a sequence-specific transcription factor that also binds to the TATA box and is highly similar to TFIID. Archaebacteria also possess a TBP homolog [5]. A signature pattern that spans the last 50 residues of the repeated region has been derived.-

Consensus pattern: Y-x-P-x(2)-[IF]-x(2)-[LIVM](2)-x-[KRH]-x(3)-P-[RKQ]-x(3)- L-[LIVM]-F-x-[STN]-G-[KR]-[LIVM]-x(3)-G-[TAGL]-[KR]-x(7)- [AGC]-x(7)-[LIVM

[1] Hoffmann A., Sinn E., Yamamoto T., Wang J., Roy A., Horikoshi M., Roeder R.G. Nature 346:387-390(1990).[2] Gash A., Hoffmann A., Horikoshi M., Roeder R.G., Chua N.-H. Nature 346:390-394(1990).[3] Nikolov D.B., Hu S.-H., Lin J., Gasch A., Hoffmann A., Horikoshi M., Chua N.-H., Roeder R.G., Burley S.K. Nature 360:40-46(1992).[4] Crowley T.E., Hoey T., Liu J.-K., Jan Y.N., Jan L.Y., Tjian R. Nature 361:557-561(1993).[5] Marsh T.L., Reich C.I., Whitelock R.B., Olsen G.J. Proc. Natl. Acad. Sci. U.S.A. 91:4180-4184(1994).

636. Translationally controlled tumor protein signatures (TCTP)

Mammalian translationally controlled tumor protein (TCTP) (or P23) is a protein which has been found to be preferentially synthesized in cells during the early growth phase of some types of tumor [1,2], but which is also expressed in normal cells. The physiological function of TCTP is still not known. It is a hydrophilic protein of 18 to 20 Kd. Close homologs have been found in plants [3], earthworm [4], Caenorhabditis elegans (F52H2.11), Hydra, budding yeast (YKL056c) [5] and fission yeast (SpAC1F12.02c) Two of the best conserved regions have been selected as signature patterns for TCTP.

Consensus pattern: [IFA]-[GA]-[GAS]-N-[PAK]-S-[GA]-E-[GDE]-[PAGE]-[DEQGA]

Consensus pattern: [FLVH]-[FY]-[IVCT]-G-E-x-[MA]-x(2,5)-[DEN]-[GAST]-x-[LV]-[AV]-x(3)-[FYW]

[1] Boehm H., Beendorf R., Gaestel M., Gross B., Nuernberg P., Kraft R., Otto A., Bielka H. Biochem. Int. 19:277-286(1989).[2] Makrides S., Chitpatima S.T., Bandyopadhyay R., Brawerman G. Nucleic Acids Res. 16:2350-2350(1988).[3] Pay A., Heberle-Bors E., Hirt H. Plant Mol. Biol. 19:501-503(1992).[4] Sturzenbaum S.R., Kille P., Morgan A.J. Biochim. Biophys. Acta 1398:294-304(1998).[5] Rasmussen S.W. Yeast 10:S63-S68(1994).

637. TFIIS zinc ribbon domain signature

Transcription factor S-II (TFIIS) [1] is a eukaryotic protein necessary for efficient RNA polymerase II transcription elongation, past template-encoded pause sites. TFIIS shows DNA-binding activity only in the presence of RNA polymerase II. It is a protein of about 300 amino acids whose sequence is highly conserved in mammals, Drosophila, yeast (where it was first known as PPR2, a transcriptional regulator of URA4, and then as DST1, the DNA strand transfer protein alpha [2]) and in the archaebacteria Sulfolobus acidocaldarius [3].This family also includes the eukaryotic and archebacterial RNA polymerase subunits of the 15 Kd / M family (see <PDOC00790>) as well as the following viral proteins: - Vaccinia virus RNA polymerase 30 Kd subunit (rpo30) [4]. - African swine fever virus protein I243L [5].The best conserved region of all these proteins contains four cysteines that bind a zinc ion and fold in a conformation termed a 'zinc ribbon' [6]. Besides these cysteines, there are a

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number of other conserved residues which can be used to help define a specific pattern for this type of domain.

Consensus pattern: C-x(2)-C-x(9)-[LIVMQSAR]-[QH]-[STQL]-[RA]-[SACR]-x-[DE]-[DET]-[PGSEA]-x(6)-C-x(2,5)-C-x(3)-[FW] [The four C's are zinc ligands]

[1] Hirashima S., Hirai H., Nakanishi Y., Natori S. J. Biol. Chem. 263:3858-3863(1988).[2] Kipling D., Kearsey S.E. Nature 353:509-509(1991).[3] Langer D., Zillig W. Nucleic Acids Res. 21:2251-2251(1993).[4] Ahn B.-Y., Gershon P.D., Jones E.V., Moss B. Mol. Cell. Biol. 10:5433-5441(1990).[5] Rodriguez J.M., Salas M.L., Vinuela E. Virology 186:40-52(1992).[6] Qian X., Jeon C., Yoon H., Agarwal K., Weiss M.A. Nature 365:277-279(1993).

638. Tetrahydrofolate dehydrogenase/cyclohydrolase signatures (THF DHG CYH)

Enzymes that participate in the transfer of one-carbon units are involved in various biosynthetic pathways. In many of these processes the transfers of one-carbon units are mediated by the coenzyme tetrahydrofolate (THF). Various reactions generate one-carbon derivatives of THF which can be interconverted between different oxidation states by formyltetrahydrofolate synthetase(EC 6.3.4.3), methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5 or EC 1.5.1.15) and methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9).The dehydrogenase and cyclohydrolase activities are expressed by a variety of multifunctional enzymes: - Eukaryotic C-1-tetrahydrofolate synthase (C1-THF synthase), which catalyzes all three reactions described above. Two forms of C1-THF synthases are known [1], one is located in the mitochondrial matrix, while the second one is cytoplasmic. In both forms the dehydrogenase/cyclohydrolase domain is located in the N-terminal section of the 900 amino acids protein and consists of about 300 amino acid residues. The C1-THF synthases are NADP- dependent. - Eukaryotic mitochondrial bifunctional dehydrogenase/cyclohydrolase [2]. This is an homodimeric NAD-dependent enzyme of about 300 amino acid residues. - Bacterial folD [3]. FolD is an homodimeric bifunctional NADP-dependent enzyme of about 290 amino acid residues. The sequence of the dehydrogenase/cyclohydrolase domain is highly conserved in all forms of the enzyme. Two conserved regions have been selected as signature patterns. The first one is located in the N-terminal part of these enzymes and contains three acidic residues. The second pattern is a highly conserved sequence of 9 amino acids which is located in the C-terminal section.

Consensus pattern: [EQ]-x-[EQK]-[LIVM](2)-x(2)-[LIVM]-x(2)-[LIVMY]-N-x-[DN]- x(5)-[LIVMF](3)-Q-L-P-[LV]

Consensus pattern: P-G-G-V-G-P-[MF]-T-[IV]

[1] Shannon K.W., Rabinowitz J.C. J. Biol. Chem. 263:7717-7725(1988).[2] Belanger C., Mackenzie R.E. J. Biol. Chem. 264:4837-4843(1989).[3] d'Ari L., Rabinowitz J.C. J. Biol. Chem. 266:23953-23958(1991).

639. Triosephosphate isomerase active site (TIM)

Triosephosphate isomerase (EC 5.3.1.1) (TIM) [1] is the glycolytic enzyme that catalyzes the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. TIM plays an important role in several metabolic pathways and is essential for efficient energy production. It is a dimer of identical subunits, each of which is made up of about 250 amino-acid residues. A glutamic acid residue is involved in the catalytic mechanism [2]. The sequence around the active site residue is perfectly conserved in all known TIM's and can be used as a signature pattern for this type of enzyme.

Consensus pattern: [AV]-Y-E-P-[LIVM]-W-[SA]-I-G-T-[GK] [E is the active site residue]

[1] Lolis E., Alber T., Davenport R.C., Rose D., Hartman F.C., Petsko G.A. Biochemistry 29:6609-6618(1990).[2] Knowles J.R. Nature 350:121-124(1991).

640. Thymidine kinase cellular-type signature (TK)

Thymidine kinase (TK) (EC 2.7.1.21) is an ubiquitous enzyme that catalyzes the ATP-dependent phosphorylation of thymidine. A comparison of TK sequences has shown [1,2,3] that there are two different families of TK. One family groups together TK from herpes viruses as well as cellular thymidylate kinases, while the second family currently consists of TK from the following sources: - Vertebrates. - Bacterial. - Bacteriophage T4. – Pox viruses. - African swine fever virus (ASF). - Fish lymphocystis disease virus (FLDV).A conserved region which is located in the C-terminal section of these enzymes has been selected as a signature pattern for this family of TKA.

Consensus pattern: [GA]-x(1,2)-[DE]-x-Y-x-[STAP]-x-C-[NKR]-x-[CH]-[LIVMFYWH]

[1] Boyle D.B., Coupar B.E.H., Gibbs A.J., Seigman L.J., Both G.W. Virology 156:355-365(1987).[2] Blasco R., Lopez-Otin C., Munoz M., Bockamp E.-O., Simon-Mateo C.,

Vinuela E. Virology 178:301-304(1990).[3] Robertson G.R., Whalley J.M. Nucleic Acids Res. 16:11303-11317(1988).

641. Thymidine kinase from herpesvirus (TK herpes)

[1]

Medline: 96003730

Crystal structures of the thymidine kinase from herpes simplex virus type-1 in complex with deoxythymidine and ganciclovir.

Brown DG, Visse R, Sandhu G, Davies A, Rizkallah PJ, Melitz C, Summers WC, Sanderson MR;

Nat Struct Biol 1995;2:876-881.

Number of members: 65

09559326.061502

642. Nuclear transition protein 2 signatures (TP2)

In mammals, the second stage of spermatogenesis is characterized by the conversion of nucleosomal chromatin to the compact, non-nucleosomal and transcriptionally inactive form found in the sperm nucleus. This condensation is associated with a double-protein transition. The first transition corresponds to the replacement of histones by several spermatid-specific proteins, also called transition proteins, which are themselves replaced by protamines during the second transition. Nuclear transition protein 2 (TP2) is one of those spermatid-specific proteins. TP2 is a basic, zinc-binding protein [1] of 116 to 137 amino-acid residues. Structurally, TP2 consists of three distinct parts: a conserved serine-rich N-terminal domain of about 25 residues, a variable central domain of 20 to 50 residues which contains cysteine residues, and a conserved C-terminal domain of about 70 residues rich in lysines and arginines. Two signature patterns for TP2 have been developed: one located in the N-terminal domain, the other in the C-terminal.

Consensus pattern: H-x(3)-H-S-[NS]-S-x-P-Q-S

Consensus pattern: K-x-R-K-x(2)-E-G-K-x(2)-K-[KR]-K

[1] Baskaran R., Rao M.R.S. Biochem. Biophys. Res. Commun. 179:1491-1499(1991).

643. Thiamine pyrophosphate enzymes signature (TTP enzymes)

A number of enzymes require thiamine pyrophosphate (TPP) (vitamin B1) as a cofactor. It has been shown [1] that some of these enzymes are structurally related. These related TPP enzymes are: - Pyruvate oxidase (POX) (EC 1.2.3.3) Reaction catalyzed: pyruvate + orthophosphate + O(2) + H(2)O = acetyl phosphate + CO(2) + H(2)O(2). - Pyruvate decarboxylase (PDC) (EC 4.1.1.1) Reaction catalyzed: pyruvate = acetaldehyde + CO(2). - Indolepyruvate decarboxylase (EC 4.1.1.74) [2] Reaction catalyzed: indole-3-pyruvate = indole-3-acetaldehyde + CO(2). - Acetolactate synthase (ALS) (EC 4.1.3.18) Reaction catalyzed: 2 pyruvate = acetolactate + CO(2). - Benzoylformate decarboxylase (BFD) (EC 4.1.1.7) [3] Reaction catalyzed: benzoylformate = benzaldehyde + CO(2). A conserved region which is located in their C-terminal section has been selected as a signature pattern for these enzymes.

Consensus pattern: [LIVMF]-[GSA]-x(5)-P-x(4)-[LIVMFYW]-x-[LIVMF]-x-G-D-[GSA]-[GSAC]

[1] Green J.B.A. FEBS Lett. 246:1-5(1989).[2] Koga J., Adachi T., Hidaka H. Mol. Gen. Genet. 226:10-16(1991).[3] Tsou A.Y., Ransom S.C., Gerlt J.A., Buechter D.D., Babbitt P.C., Kenyon G.L. Biochemistry 29:9856-9862(1990).

09555356.052001

644. TPR Domain

[1]

Medline: 95397415

Tetratrico peptide repeat interactions: to TPR or not to TPR?

Lamb JR, Tugendreich S, Hieter P;

Trends Biochem Sci 1995;20:257-259.

[2]Medline: 98151343

The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions.

Das AK, Cohen PW, Barford D;

EMBO J 1998;17:1192-1199.

Number of members: 621

645. Uroporphyrin-III C-methyltransferase signatures (TP methylase)

Uroporphyrin-III C-methyltransferase (EC 2.1.1.107) (SUMT) [1,2] catalyzes the transfer of two methyl groups from S-adenosyl-L-methionine to the C-2 and C-7atoms of uroporphyrinogen III to yield precorrin-2 via the intermediate formation of precorrin-1. SUMT is the first enzyme specific to the cobalamin pathway and precorrin-2 is a common intermediate in the biosynthesis of corrinoids such as vitamin B12, siroheme and coenzyme F430.The sequences of SUMT from a variety of eubacterial and archaebacterial species are currently available. In species such as Bacillus megaterium (gene cobA), Pseudomonas denitrificans (cobA) or Methanobacterium ivanovii (gene corA) SUMT is a protein of about 25 to 30 Kd. In Escherichia coli and related bacteria, the cysG protein, which is involved in the biosynthesis of siroheme, is a multifunctional protein composed of a N-terminal domain, probably involved in transforming precorrin-2 into siroheme, and a C-terminal domain which has SUMT activity. The sequence of SUMT is related to that of a number of P. denitrificans and Salmonella typhimurium enzymes involved in the biosynthesis of cobalamin which also seem to be SAM-dependent methyltransferases [3,4]. The similarity is especially strong with two of these enzymes: cobI/cbiL which encodes S-adenosyl-L-methionine--precorrin-2 methyltransferase and cobM/cbiF whose exact function is not known. Two signature patterns have been developed for these enzymes. The first corresponds to a well conserved region in the N-terminal extremity (called region 1 in [1,3]) and the second to a less conserved region located in the central part of these proteins (this pattern spans what are called regions 2 and 3 in [1,3]).

Consensus pattern: [LIVM]-[GS]-[STAL]-G-P-G-x(3)-[LIVMFY]-[LIVM]-T-[LIVM]-[KRHQG]-[AG]

Consensus pattern: V-x(2)-[LI]-x(2)-G-D-x(3)-[FYW]-[GS]-x(8)-[LIVF]-x(5,6)-[LIVMFYWPAC]-x-[LIVMY]-x-P-G

[1] Blanche F., Robin C., Couder M., Faucher D., Cauchois L., Cameron B., Crouzet J. J. Bacteriol. 173:4637-4645(1991).[2] Robin C., Blanche F., Cauchois L., Cameron B., Couder M., Crouzet J. J. Bacteriol. 173:4893-4896(1991).[3] Crouzet J., Cameron B., Cauchois L., Rigault S., Rouyez M.-C., Blanche F., Thibaut D., Debussche L. J. Bacteriol. 172:5980-5990(1990).[4] Roth J.R., Lawrence J.G., Rubenfield M., Kieffer-Higgins S., Church G.M. J.

Bacteriol. 175:3303-3316(1993).[5] Mattheakis L.C., Shen W.H., Collier R.J. Mol. Cell. Biol. 12:4026-4037(1992).

646. Tudor domain

Domain of unknown function present in several RNA-binding proteins. copies in the Drosophila Tudor protein. Slight ambiguities in the alignment.Number of members: 18 [1]Medline: 97200561 Tudor domains in proteins that interact with RNA. Ponting CP; Trends Biochem Sci 1997;22:51-52. [2]Medline: 97157029 The human EBNA-2 coactivator p100: multidomain organization and relationship to the staphylococcal nuclease fold and to the tudor protein involved in Drosophila melanogaster development. Callebaut I, Mornon JP; Biochem J 1997;321:125-132.

647. Terpene synthase family

It has been suggested that this gene family be designated tps (for terpene synthase) [1]. It has been split into six subgroups on the basis of phylogeny, called tpsa-tpsf.

tpsa includes vetispiridiene synthase Swiss:Q39979, 5-epi-aristolochene synthase, Swiss:Q40577 and (+)-delta-cadinene synthase Swiss:P93665.

tpsb includes (-)-limonene synthase, Swiss:Q40322.

tpsc includes kaurene synthase A, Swiss:O04408.

tpsd includes taxadiene synthase, Swiss:Q41594, pinene synthase, Swiss:O24475 and myrcene synthase, Swiss:O24474.

tpse includes kaurene synthase B.

tpsf includes linalool synthase.

Number of members: 51

[1]

Medline: 97413772

Monoterpene synthases from grand fir (Abies grandis). cDNA isolation, characterization, and functional expression of myrcene synthase, (-)-(4S)-limonene synthase, and

(-)-(1S,5S)-pinene synthase.
Bohlmann J, Steele CL, Croteau R;
J Biol Chem 1997;272:21784-21792.

648. ThiF family

This family contains a repeated domain in ubiquitin activating enzyme E1 and members of the bacterial ThiF/MoeB/HesA family.Number of members: 87

649. Thioester dehydrase

Members of this family are involved in fatty acid biosynthesis.
Number of members: 19
[1]
Medline: 96398612
Structure of a dehydratase-isomerase from the bacterial pathway for biosynthesis of unsaturated fatty acids: two catalytic activities in one active site.
Leesong M, Henderson BS, Gillig JR, Schwab JM, Smith JL;
Structure 1996;4:253-264.
Database Reference: SCOP; 1mka; fa; [SCOP-USA][CATH-PDBSUM]
Database reference: PFAMB; PB058036;

650. Tub family signatures

The mouse tubby mutation is the cause of maturity-onset obesity, insulin resistance and sensory deficits. This mutation maps to a gene, tub [1,2],which codes for a protein that belongs to a family which currently consists of the following members: - Mammalian tub, an hydrophilic protein of about 500 residues, which could be involved in the hypothalamic regulation of body weight. - Human protein TULP1 [3] which may be involved in retinis pigmentosa 14, a retinal degeneration disease. - Mouse protein p4-6 whose function is not known. - Caenorhabditis elegans hypothetical protein F10B5.4. - Several fragmentary

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sequences from plants, Drosophila and human ESTs. While the N-terminal part of these protein is not conserved in length nor in the sequence, the C-terminal 250 residues are highly conserved. Therefore, two regions were selected in the C-terminal part as signature patterns. The secondr egion is located at the C-terminal extremity and contains a penultimate cysteine residue that could be critical to the normal functioning of these proteins.

Consensus pattern: F-[KHQ]-G-R-V-[ST]-x-A-S-V-K-N-F-Q

Consensus pattern: A-F-[AG]-I-[SAC]-[LIVM]-[ST]-S-F-x-[GST]-K-x-A-C-E

[1] Kleyn P.W., Fan W., Kovats S.G., Lee J.L., Pulido J.C., Wu Y., Berkemeier L.R., Misumi D.J., Holmgren L., Charlat O., Woolf E.A., Tayber O., Brody T., Shu P., Hawkins F., Kennedy B., Baldini L., Ebeling C., Alperin G.D., Deeds J., Lakey N.D., Culpepper J., Chen H., Gluecksmann-Kuis M.A., Carlson G.A., Duyk G.M., Moore K.J. Cell 85:281-290(1996).[2] Noben-Trauth K., Naggert J.K., North M.A., Nishina P.M. Nature 380:534-538(1996).[3] North M.A., Naggert J.K., Yan Y., Noben-Trauth K., Nishina P.M. Proc. Natl. Acad. Sci. U.S.A. 94:3128-3133(1997).

09535375.061700

651. Eukaryotic DNA topoisomerase I active site

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type Itopoisomerases act by catalyzing the transient breakage of DNA, one strand at a time, and the subsequent rejoining of the strands. When a eukaryotic type 1topoisomerase breaks a DNA backbone bond, it simultaneously forms a protein-DNA link where the hydroxyl group of a tyrosine residue is joined to a 3'-phosphate on DNA, at one end of the enzyme-severed DNA strand. In eukaryotes and pox virus topoisomerases I, there are a number of conserved residues in the region around the active site tyrosine.

Consensus pattern: [DEN]-x(6)-[GS]-[IT]-S-K-x(2)-Y-[LIVM]-x(3)-[LIVM] [Y is the active site tyrosine]

[1] Sternglanz R. Curr. Opin. Cell Biol. 1:533-535(1990).[2] Sharma A., Mondragon A. Curr. Opin. Struct. Biol. 5:39-47(1995).[3] Lynn R.M., Bjornsti M.-A., Caron P.R., Wang J.C. Proc. Natl. Acad. Sci. U.S.A. 86:3559-3563(1989).[4] Roca J. Trends Biochem. Sci. 20:156-160(1995).[E1]

652. Transaldolase signatures

Transaldolase (EC 2.2.1.2) catalyzes the reversible transfer of a three-carbonketol unit from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to form erythrose 4-phosphate and fructose 6-phosphate. This enzyme, together with transketolase, provides a link between the glycolytic and pentose-phosphate pathways. Transaldolase is an enzyme of about 34 Kd whose sequence has been well conserved throughout evolution. A lysine has been implicated [1]in the catalytic mechanism of the enzyme; it acts as a nucleophilic group that attacks the carbonyl group of fructose-6-phosphate.Transaldolase is evolutionary related [2] to a bacterial protein of about 20Kd (known as talC in Escherichia coli), whose exact function is not yet known. Two signature patterns have been developed for these proteins. The first, located in the N-terminal section, contains a perfectly conserved pentapeptide; these cond, includes the active site lysine.

Consensus pattern: [DG]-[IVSA]-T-[ST]-N-P-[STA]-[LIVMF](2)

Consensus pattern: [LIVM]-x-[LIVM]-K-[LIVM]-[PAS]-x-[ST]-x-[DENQPAS]-G- [LIVM]-x-[AGV]-x-[QEKRST]-x-[LIVM] [K is the active site residue]

[1] Miosga T., Schaaff-Gerstenschlaeger I., Franken E., Zimmermann F.K. Yeast 9:1241-1249(1993).[2] Reizer J., Reizer A., Saier M.H. Jr. Microbiology 141:961-971(1995).

653. (Transpeptidase) Penicillin binding protein transpeptidase domain

The active site serine (residue 337 in Swiss:P14677) is conserved in all members of this family.

[1] Pares S, Mouz N, Petillot Y, Hakenbeck R, Dideberg O Nat Struct Biol 1996;3:284-289.

654. Trehalase signatures

Trehalase (EC 3.2.1.28) is the enzyme responsible for the degradation of the disaccharide alpha, alpha-trehalose yielding two glucose subunits [1]. It is an enzyme found in a wide variety of organisms and whose sequence has been highly conserved throughout evolution. Two of the most highly conserved regions have been selected as signature patterns. The first pattern is located in the central section, the second one is in the C-terminal region.

Consensus pattern: P-G-G-R-F-x-E-x-Y-x-W-D-x-Y

Consensus pattern: Q-W-D-x-P-x-[GA]-W-[PAS]-P

[1] Kopp M., Mueller H., Holzer H. J. Biol. Chem. 268:4766-4774(1993).[2] Henrissat B., Bairoch A. Biochem. J. 293:781-788(1993).[E1]

655. Trehalose-6-phosphate synthase domain

OtsA (Trehalose-6-phosphate synthase) is homologous to regions in the subunits of yeast trehalose-6-phosphate synthase/phosphate complex, [1].

[1] Kaasen I, McDougall J, Strom AR; Gene 1994;145:9-15.

09595352.052500

656. Tropomyosins signature

Tropomyosins [1,2] are family of closely related proteins present in muscle and non-muscle cells. In striated muscle, tropomyosin mediate the interactions between the troponin complex and actin so as to regulate muscle contraction. The role of tropomyosin in smooth muscle and non-muscle tissues is not clear. Tropomyosin is an alpha-helical protein that forms a coiled-coil dimer. Muscle isoforms of tropomyosin are characterized by having 284 amino acid residues and a highly conserved N-terminal region, whereas non-muscle forms are generally smaller and are heterogeneous in their N-terminal region. The signature pattern for tropomyosins is based on a very conserved region in the C-terminal section of tropomyosins and which is present in both muscle and non-muscle forms.

Consensus pattern: L-K-E-A-E-x-R-A-E

[1] Smilie L.B. Trends Biochem. Sci. 4:151-155(1979).[2] McLeod A.R. BioEssays 6:208-212(1986).

657. Troponin

Troponin (Tn) contains three subunits, Ca2+ binding (TnC), inhibitory (TnI), and tropomyosin binding (TnT). this Pfam contains members of the TnT subunit.

Troponin is a complex of three proteins, Ca2+ binding (TnC), inhibitory (TnI), and tropomyosin binding (TnT).

The troponin complex regulates Ca++ induced muscle contraction. This family includes troponin T and troponin I. Troponin I binds to actin and troponin T binds to tropomyosin.

Number of members: 81 [1]

Medline: 87144593

Structure of co-crystals of tropomyosin and troponin.

White SP, Cohen C, Phillips GN Jr;

Nature 1987;325:826-828. [2]

Medline: 95155315

A direct regulatory role for troponin T and a dual role for troponin C in the Ca2+ regulation of muscle contraction.

Potter JD, Sheng Z, Pan BS, Zhao J;

J Biol Chem 1995;270:2557-2562.

[3]Medline: 95324796

The troponin complex and regulation of muscle contraction.

Farah CS, Reinach FC;

FASEB J 1995;9:755-767.

09525369.062502

658. (Tryp mucin) Mucin-like glycoprotein

This family of trypanosomal proteins resemble vertebrate mucins. The protein consists of three regions. The N and C terminii are conserved between all members of the family, whereas the central region is not well conserved and contains a large number of threonine residues which can be glycosylated [1].

Indirect evidence suggested that these genes might encode the core protein of parasite mucins, glycoproteins that were proposed to be involved in the interaction with, and invasion of, mammalian host cells.

[1] Di Noia JM, Sanchez DO, Frasch AC; J Biol Chem 1995;270:24146-24149.

[2] Di Noia JM, D'Orso I, Aslund L, Sanchez DO, Frasch AC; J Biol Chem 1998;273:10843-10850.

659. Aminoacyl-transfer RNA synthetases class-I signature (tRNA synt 1)

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each differentamino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse interms of subunit size and of quaternary structure. A few years ago it was found [2] that several aminoacyl-tRNA synthetases share a region of similarity in their N-terminal section, in particular the consensus tetrapeptide His-Ile-Gly-His ('HIGH') is very well conserved. The 'HIGH' region has been shown [3] to be part of the adenylate binding site. The 'HIGH' signature has been found in the aminoacyl-tRNA synthetases specific for arginine, cysteine, glutamic acid, glutamine, isoleucine, leucine, methionine, tyrosine, tryptophan, and valine. These aminoacyl-tRNA synthetases are referred to as class-I synthetases [4,5,6] and seem to share the same tertiarystructure based on a Rossmann fold.

Consensus pattern: P-x(0,2)-[GSTAN]-[DENQGAPK]-x-[LIVMFP]-[HT]-[LIVMYAC]-G-[HNTG]-[LIVMFYSTAGPC]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Webster T., Tsai H., Kula M., Mackie G.A., Schimmel P. Science 226:1315-1317(1984).[3] Brick P., Bhat T.N., Blow D.M. J. Mol. Biol. 208:83-98(1988).[4] Delarue M., Moras D. BioEssays 15:675-687(1993).[5] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[6] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

660. Aminoacyl-transfer RNA synthetases class-I signature (tRNA synt 1b)

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. A few years ago it was found [2]

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that several aminoacyl-tRNA synthetases share a region of similarity in their N-terminal section, in particular the consensus tetrapeptide His-Ile-Gly-His ('HIGH') is very well conserved. The 'HIGH' region has been shown [3] to be part of the adenylate binding site. The 'HIGH' signature has been found in the aminoacyl-tRNA synthetases specific forarginine, cysteine, glutamic acid, glutamine, isoleucine, leucine, methionine, tyrosine, tryptophan, and valine. These aminoacyl-tRNA synthetases are referred to as class-I synthetases [4,5,6] and seem to share the same tertiary structure based on a Rossmann fold. Consensus pattern: P-x(0,2)-[GSTAN]-[DENQGAPK]-x-[LIVMFP]-[HT]-[LIVMYAC]-G-[HNTG]-[LIVMFYSTAGPC

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Webster T., Tsai H., Kula M., Mackie G.A., Schimmel P. Science 226:1315-1317(1984).[3] Brick P., Bhat T.N., Blow D.M. J. Mol. Biol. 208:83-98(1988).[4] Delarue M., Moras D. BioEssays 15:675-687(1993).[5] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[6] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

661. (tRNA-synt 1C) tRNA synthetases class I (E and Q)

Other tRNA synthetase sub-families are too dissimilar to be included.
This family includes only glutamyl and glutaminyl tRNA synthetases.
In some organisms, a single glutamyl-tRNA synthetase aminoacylates both tRNA(Glu) and tRNA(Gln).

[1] Rath VL, Silvian LF, Beijer B, Sproat BS, Steitz TA; Structure 1998;6:439-449.

662. (tRNA-synt 1d) tRNA synthetases class I (R)

Other tRNA synthetase sub-families are too dissimilar to be included.
This family includes only arginyl tRNA synthetase.

663. Aminoacyl-transfer RNA synthetases class-II signatures (tRNA synt 2)

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse interms of subunit size and of quaternary structure. The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7].Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns have been derived from two of these regions.

Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE

Consensus pattern: [GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Delarue M., Moras D. BioEssays 15:675-687(1993).[3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[4] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991). [5] Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).[6] Cusack S. Biochimie 75:1077-1081(1993).[7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990).[8] Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

664. Aminoacyl-transfer RNA synthetases class-I signature (tRNA synt 1e)

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. A few years ago it was found [2] that several aminoacyl-tRNA synthetases share a region of similarity in their N-terminal section, in particular the consensus tetrapeptide His-Ile-Gly-His ('HIGH') is very well conserved. The 'HIGH' region has been shown [3] to be part of the adenylate binding site. The 'HIGH' signature has been found in the aminoacyl-tRNA synthetases specific forarginine, cysteine, glutamic acid, glutamine, isoleucine, leucine, methionine, tyrosine, tryptophan, and valine. These aminoacyl-tRNA synthetases are referred to as class-I synthetases [4,5,6] and seem to share the same tertiary structure based on a Rossmann fold. Consensus pattern: P-x(0,2)-[GSTAN]-[DENQGAPK]-x-[LIVMFP]-[HT]-[LIVMYAC]-G-[HNTG]-[LIVMFYSTAGPC

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Webster T., Tsai H., Kula M., Mackie G.A., Schimmel P. Science 226:1315-1317(1984).[3] Brick P., Bhat T.N., Blow D.M. J. Mol. Biol. 208:83-98(1988).[4] Delarue M., Moras D. BioEssays 15:675-687(1993).[5] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[6] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

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665. Aminoacyl-transfer RNA synthetases class-II signatures (tRNA synt 2b)

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse interms of subunit size and of quaternary structure. The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7].Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns have been derived from two of these regions.

Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE

Consensus pattern: [GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Delarue M., Moras D. BioEssays 15:675-687(1993).[3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[4] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991). [5] Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).[6] Cusack S. Biochimie 75:1077-1081(1993).[7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990).[8] Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

666. Thaumatin family signature

Thaumatin [1] is an intensively sweet-tasting protein (100 000 times sweeter than sucrose on a molar basis) from Thaumatococcus daniellii, an African brush. The protein is made of about 200 residues and contains 8 disulfide bonds. A number of proteins have been found to be related to thaumatins. These protein are listed below (references are only provided for recently determined sequences). - A maize alpha-amylase/trypsin inhibitor. - Two tobacco pathogenesis-related proteins: PR-R major and minor forms, which are induced after infection with viruses. - Salt-induced protein NP24 from tomato. - Osmotin, a salt-induced protein from tobacco. - Osmotin-like proteins OSML13, OSML15 and OSML81 from potato [2]. - P21, a leaf protein from soybean. - PWIR2, a leaf protein from wheat. - Zeamatin, a maize antifunal protein [3].The exact biological function of all these proteins is not yet known. A conserved region that includes three cysteine residues known (in thaumatin) to be involved in disulfide bonds has been selected as a signature pattern.

+--+ | +-----------------+ | | ******* | | |

xxCxxxxxxxxxxxxxxxxCxxCxxCxCxxxxxxxxxxxxxxCxxCxCxxxCxCxxCCxCxxxCxxxxxC xxxCx | | | | | | | | || | | +--+ +-+ | +----+ +--++-+ | +-------------------------+'C': conserved cysteine involved in a disulfide bond.'*': position of the pattern.

Consensus pattern: G-x-[GF]-x-C-x-T-[GA]-D-C-x(1,2)-G-x(2,3)-C

[1] Edens L., Heslinga L., Klok R., Ledeboer A.M., Maat J., Toonen M.Y., Visser C., Verrips C.T. Gene 18:1-12(1982).[2] Zhu B., Chen T.H.H., Li P.H. Plant Physiol. 108:929-937(1995).[3] Malehorn D.E., Borgmeyer J.R., Smith C.E., Shah D.M.; Plant Physiol. 106:1471-1481(1994).

667. Thiolases signatures

Two different types of thiolase [1,2,3] are found both in eukaryotes and in prokaryotes: acetoacetyl-CoA thiolase (EC 2.3.1.9) and 3-ketoacyl-CoA thiolase(EC 2.3.1.16). 3-ketoacyl-CoA thiolase (also called thiolase I) has a broad chain-length specificity for its substrates and is involved in degradative pathways such as fatty acid beta-oxidation. Acetoacetyl-CoA thiolase (also called thiolase II) is specific for the thiolysis of acetoacetyl-CoA and involved in biosynthetic pathways such as poly beta-hydroxybutyrate synthesisor steroid biogenesis. In eukaryotes, there are two forms of 3-ketoacyl-CoA thiolase: one located in the mitochondrion and the other in peroxisomes. There are two conserved cysteine residues important for thiolase activity. The first located in the N-terminal section of the enzymes is involved in the formation of an acyl-enzyme intermediate; the second located at the C-terminal extremity is the active site base involved in deprotonation in the condensation reaction. Mammalian nonspecific lipid-transfer protein (nsL-TP) (also known as sterol carrier protein 2) is a protein which seems to exist in two different forms: a 14 Kd protein (SCP-2) and a larger 58 Kd protein (SCP-x). The former is found in the cytoplasm or the mitochondria and is involved in lipid transport; the latter is found in peroxisomes. The C-terminal part of SCP-x is identical to SCP-2 while the N-terminal portion is evolutionary related to thiolases[4]. Three signature patterns have been developed for this family of proteins, two of which are based on the regions around the biologically important cysteines. The third is based on a highly conserved region in the C-terminal part of these proteins.

Consensus pattern: [LIVM]-[NST]-x(2)-C-[SAGLI]-[ST]-[SAG]-[LIVMFYNS]-x- [STAG]-[LIVM]-x(6)-[LIVM] [C is involved in formation of acyl-enzyme intermediate]

Consensus pattern: N-x(2)-G-G-x-[LIVM]-[SA]-x-G-H-P-x-[GA]-x-[ST]-G

Consensus pattern: [AG]-[LIVMA]-[STAGCLIVM]-[STAG]-[LIVMA]-C-x-[AG]-x-[AG]-x- [AG]-x-[SAG] [C is the active site residue]

[1] Peoples O.P., Sinskey A.J. J. Biol. Chem. 264:15293-15297(1989).[2] Yang S.-Y., Yang X.-Y.H., Healy-Louie G., Schulz H., Elzinga M. J. Biol. Chem. 265:10424-10429(1990).[3] Igual J.C., Gonzalez-Bosch C., Dopazo J., Perez-Ortin J.E. J. Mol. Evol. 35:147-155(1992).[4] Baker M.E., Billheimer J.T., Strauss J.F. III DNA Cell Biol. 10:695-698(1991).

668. Thioredoxin family active site

Thioredoxins [1 to 4] are small proteins of approximately one hundred amino-acid residues which participate in various redox reactions via the reversible oxidation of an active center disulfide bond. They exist in either a reduced form or an oxidized form where the two cysteine residues are linked in an intramolecular disulfide bond. Thioredoxin is present in prokaryotes and eukaryotes and the sequence around the redox-active disulfide bond is wellconserved. Bacteriophage T4 also encodes for a thioredoxin but its primary structure is not homologous to bacterial, plant and vertebrate thioredoxins. A number of eukaryotic proteins contain domains evolutionary related tothioredoxin, all of them seem to be protein disulphide isomerases (PDI). PDI(EC 5.3.4.1) [5,6,7] is an endoplasmic reticulum enzyme that catalyzes the rearrangement of disulfide bonds in various proteins. The various forms of PDI which are currently known are: - PDI major isozyme; a multifunctional protein that also function as the beta subunit of prolyl 4-hydroxylase (EC 1.14.11.2), as a component of oligosaccharyl transferase (EC 2.4.1.119), as thyroxine deiodinase (EC 3.8. 1.4), as glutathione-insulin transhydrogenase (EC 1.8.4.2) and as a thyroid hormone-binding protein ! - ERp60 (ER-60; 58 Kd microsomal protein). ERp60 was originally thought to be a phosphoinositide-specific phospholipase C isozyme and later to be a protease. - ERp72. - P5.All PDI contains two or three (ERp72) copies of the thioredoxin domain. Bacterial proteins that act as thiol:disulfide interchange proteins thatallows disulfide bond formation in some periplasmic proteins also contain a thioredoxin domain. These proteins are: - Escherichia coli dsbA (or prfA) and its orthologs in Vibrio cholerae (tcpG) and Haemophilus influenzae (por). - Escherichia coli dsbC (or xpRA) and its orthologs in Erwinia chrysanthemi and Haemophilus influenzae. - Escherichia coli dsbD (or dipZ) and its Haemophilus influenzae ortholog. - Escherichia coli dsbE (or ccmG) and orthologs in Haemophilus influenzae, Rhodobacter capsulatus (helX), Rhiziobiacae (cycY and tlpA).

Consensus pattern: [LIVMF]-[LIVMSTA]-x-[LIVMFYC]-[FYWSTHE]-x(2)-[FYWGTN]-C- [GATPLVE]-[PHYWSTA]-C-x(6)-[LIVMFYWT] [The two C's form the redox-active bond]

[1] Holmgren A. Annu. Rev. Biochem. 54:237-271(1985).[2] Gleason F.K., Holmgren A. FEMS Microbiol. Rev. 54:271-297(1988).[3] Holmgren A. J. Biol. Chem. 264:13963-13966(1989).[4] Eklund H., Gleason F.K., Holmgren A. Proteins 11:13-28(1991).[5] Freedman R.B., Hawkins H.C., Murant S.J., Reid L. Biochem. Soc. Trans. 16:96-99(1988).[6] Kivirikko K.I., Myllyla R., Pihlajaniemi T. FASEB J. 3:1609-1617(1989).[7] Freedman R.B., Hirst T.R., Tuite M.F. Trends Biochem. Sci. 19:331-336(1994).

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669. (Transcript fac2) Transcription factor TFIIB repeat signature

In eukaryotes the initiation of transcription of protein encoding genes by polymerase II is modulated by general and specific transcription factors. The general transcription factors operate through common promoters elements (such as the TATA box). At least seven different proteins associates to form the general transcription factors: TFIIA, -IIB, -IID, -IIE, -IIF, -IIG, and -IIH[1].Transcription factor IIB (TFIIB) plays a central role in the transcription of class II genes, it associates with a complex of TFIID-IIA bound to DNA (DA complex) to form a ternary complex TFIID-IIA-IBB (DAB complex) which is then recognized by RNA polymerase II [2,3]. TFIIB is a protein of about 315 to 340amino acid residues which contains, in its C-terminal part an imperfect repeat of a domain of about 75 residues. This repeat could contribute an element of symmetry to the folded protein. The following proteins have been shown to be evolutionary related to TFIIB: - An archaebacterial TFIIB homolog. In Pyrococcus woesei a previously undetected open reading frame has been shown [4] to be highly related to TFIIB. - Fungal transcription factor IIIB 70 Kd subunit (gene PCF4/TDS4/BRF1) [5]. This protein is a general activator of RNA polymerase III transcription and plays a role analogous to that of TFIIB in pol III transcription. The central section of the repeated domain, which is the most conserved part of that domain has been selected as a signature pattern.

Consensus pattern: G-[KR]-x(3)-[STAGN]-x-[LIVMYA]-[GSTA](2)-[CSAV]-[LIVM]-[LIVMFY]-[LIVMA]-[GSA]-[STAC

[1] Weinmann R. Gene Expr. 2:81-91(1992).[2] Hawley D. Trends Biochem. Sci. 16:317-318(1991).[3] Ha I., Lane W.S., Reinberg D. Nature 352:689-695(1991).[4] Ouzounis C., Sander C. Cell 71:189-190(1992).[5] Khoo B., Brophy B., Jackson S.P. Genes Dev. 8:2879-2890(1994).

670. (transcritp fact) MADS-box domain signature and profile

A number of transcription factors contain a conserved domain of 56 amino-acid residues, sometimes known as the MADS-box domain [E1]. They are listed below: - Serum response factor (SRF) [1], a mammalian transcription factor that binds to the Serum Response Element (SRE). This is a short sequence of dyad symmetry located 300 bp to the 5' end of the transcription initiation site of genes such as c-fos. - Mammalian myocyte-specific enhancer factors 2A to 2D (MEF2A to MEF2D). These proteins are transcription factor which binds specifically to the MEF2 element present in the regulatory regions of many muscle-specific genes. - Drosophila myocyte-specific enhancer factor 2 (MEF2). - Yeast GRM/PRTF protein (gene MCM1) [2], a transcriptional regulator of mating-type-specific genes. - Yeast arginine metabolism regulation protein I (gene ARGR1 or ARG80). - Yeast transcription factor RLM1. - Yeast transcription factor SMP1. - Arabidopsis thaliana agamous protein (AG) [3], a probable transcription factor involved in regulating genes that determines stamen and carpel development in wild-type flowers. Mutations in the AG gene result in the replacement of the stamens by petals and the carpels by a new flower. - Arabidopsis thaliana homeotic proteins Apetala1 (AP1), Apetala3 (AP3) and Pistillata (PI) which act locally to specify the identity of the floral meristem and to determine sepal and petal development [4]. - Antirrhinum majus and tobacco homeotic protein deficiens (DEFA) and globosa (GLO) [5]. Both proteins are transcription factors involved in the genetic control of flower development. Mutations in DEFA or GLO cause the transformation of petals into sepals and of stamina into carpels. - Arabidopsis thaliana putative transcription factors AGL1 to AGL6 [6]. - Antirrhinum majus morphogenetic protein DEF H33 (squamosa).In SRF, the conserved domain has been shown [1] to be involved in DNA-binding and dimerization. A pattern that spans the complete length of the domain has been derived. The profile also spans the length of the MADS-box.

Consensus pattern: R-x-[RK]-x(5)-I-x-[DNGSK]-x(3)-[KR]-x(2)-T-[FY]-x-[RK](3)- x(2)-[LIVM]-x-K(2)-A-x-E-[LIVM]-[STA]-x-L-x(4)-[LIVM]-x- [LIVM](3)-x(6)-[LIVMF]-x(2)-[FY]

[1] Norman C., Runswick M., Pollock R., Treisman R. Cell 55:989-1003(1988).[2] Passmore S., Maine G.T., Elble R., Christ C., Tye B.-K. J. Mol. Biol. 204:593-606(1988).[3] Yanofsky M., Ma H., Bowman J., Drews G., Feldmann K.A., Meyerowitz E.M. Nature 346:35-39(1990).[4] Goto K., Meyerowitz E.M. Genes Dev. 8:1548-1560(1994).[5] Troebner W., Ramirez L., Motte P., Hue I., Huijser P., Loennig W.-E., Saedler H., Sommer H., Schwartz-Sommer Z. EMBO J. 11:4693-4704(1992).[6] Ma H., Yanofsky M.F., Meyerowitz E.M. Genes Dev. 5:484-495(1991).[E1]

671. Transketolase signatures

Transketolase (EC 2.2.1.1) (TK) catalyzes the reversible transfer of a two-carbon ketol unit from xylulose 5-phosphate to an aldose receptor, such as ribose 5-phosphate, to form sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate. This enzyme, together with transaldolase, provides a link between the glycolytic and pentose-phosphate pathways. TK requires thiamin pyrophosphate as a cofactor. In most sources where TK has been purified, it is a homodimer of approximately 70 Kd subunits. TK sequences from a variety of eukaryotic and prokaryotic sources [1,2] show that the enzyme has been evolutionarily conserved. In the peroxisomes of methylotrophic yeast Hansenula polymorpha, there is a highly related enzyme, dihydroxy-acetone synthase (DHAS) (EC 2.2.1.3) (also known as formaldehyde transketolase), which exhibits a very unusual specificity by including formaldehyde amongst its substrates. 1-deoxyxylulose-5-phosphate synthase (DXP synthase) [3] is an enzyme so far found in bacteria (gene dxs) and plants (gene CLA1) which catalyzes the thiamin pyrophosphoate-dependent acyloin condensation reaction between carbon atoms 2 and 3 of pyruvate and glyceraldehyde 3-phosphate to yield 1-deoxy-D- xylulose-5-phosphate (dxp), a precursor in the biosynthetic pathway to isoprenoids, thiamin (vitamin B1), and pyridoxol (vitamin B6). DXP synthase is evolutionary related to TK. Two regions of TK have been selected as signature patterns. The first, located in the N-terminal section, contains a histidine residue which appears to function inproton transfer during catalysis [4]. The second, located in the central section, contains conserved acidic residues that are part of the active cleft and may participate in substrate-binding [4].

Consensus pattern: R-x(3)-[LIVMTA]-[DENQSTHKF]-x(5,6)-[GSN]-G-H-[PLIVMF]-[GSTA]-x(2)-[LIMC]-[GS

Consensus pattern: G-[DEQGSA]-[DN]-G-[PAEQ]-[ST]-[HQ]-x-[PAGM]-[LIVMYAC]-[DEFYW]-x(2)-[STAP]-x(2)-[RGA]

[1] Abedinia M., Layfield R., Jones S.M., Nixon P.F., Mattick J.S. Biochem. Biophys. Res. Commun. 183:1159-1166(1992).[2] Fletcher T.S., Kwee I.L., Nakada T., Largman C., Martin B.M. Biochemistry 31:1892-1896(1992).[3] Sprenger G.A., Schorken U., Wiegert T., Grolle S., De Graaf A.A., Taylor S.V., Begley T.P., Bringer-Meyer S., Sahm H. Proc. Natl. Acad. Sci. U.S.A. 94:12857-12862(1997).[4] Lindqvist Y., Schneider G., Ermler U., Sundstroem M. EMBO J. 11:2373-2379(1992).

672. Transmembrane 4 family signature

Recently a number of eukaryotic cell surface antigens have been found to be evolutionary related [1,2,3]. The proteins known to belong to this family are listed below: - Mammalian antigen CD9 (MIC3); A protein involved in platelet activation and aggregation. - Mammalian leukocyte antigen CD37, expressed on B lymphocytes. - Mammalian leukocyte antigen CD53 (OX-44), which may be involved in growth regulation in hematopoietic cells. - Mammalian lysosomal membrane protein CD63 (melanoma-associated antigen ME491; antigen AD1). - Mammalian antigen CD81 (cell surface protein TAPA-1), which may play an important role in the regulation of lymphoma cell growth. - Mammalian antigen CD82 (protein R2; antigen C33; Kangai 1 (KAI1)), which associates with CD4 or CD8 and delivers costimulatory signals for the TCR/CD3 pathway. - Mammalian antigen CD151 (SFA-1; platelet-endothelial tetraspan antigen 3 (PETA-3)). - Mammalian cell surface glycoprotein A15 (TALLA-1; MXS1). - Mammalian novel antigen 2 (NAG-2). - Human tumor-associated antigen CO-029. - Schistosoma mansoni and japonicum 23 Kd surface antigen (SM23 / SJ23).These proteins share the following characteristics: they all seem to be type III membrane proteins (type III proteins are integral membrane proteins that contain a N-terminal membrane-anchoring domain which is not cleaved during biosynthesis and which functions both as a translocation signal and as a membrane anchor); they also contain three additional transmembrane regions, at least seven conserved cysteines residues, and are of approximately the same size (218 to 284 residues). These proteins are collectively know as the 'transmembrane 4 super family' (TM4) because they span the plasma membrane four times. A schematic diagram of the domain structure of these proteins isshown below. +-+-----+-------+----+-----+-----+------------ ----------------+-----+----+ | | TMa | Extra | TM2| Cyt | TM3 | Extracellular | TM4 | Cyt| +-+----- +-------+----C-----C-----+--------CC---------C-----C---+-----C----+ ********Cyt : cytoplasmic domain. TMa : transmembrane anchor.TM2 to TM4: transmembrane regions 2 to 4.'C' : conserved cysteine. '*' : position of the pattern.

A conserved region that includes two cysteines and seems to be located in a short cytoplasmic loop between two transmembrane domains has been selected as a signature for these proteins.

Consensus pattern: G-x(3)-[LIVMF]-x(2)-[GSA]-[LIVMF](2)-G-C-x-[GA]-[STA]- x(2)-[EG]-x(2)-[CWN]-[LIVM](2)

[1] Levy S., Nguyen V.Q., Andria M.L., Takahashi S. J. Biol. Chem. 266:14597-14602(1991).[2] Tomlinson M.G., Williams A.F., Wright M.D. Eur. J. Immunol. 23:136-40(1993).[3] Barclay A.N., Birkeland M.L., Brown M.H., Beyers A.D., Davis S.J., Somoza

C., Williams A.F. The leucocyte antigen factbooks. Academic Press, London / San Diego, (1993).

673. Tryptophan synthase alpha chain signature

Tryptophan synthase catalyzes the last step in the biosynthesis of tryptophan: the conversion of indoleglycerol phosphate and serine, totryptophan and glyceraldehyde 3-phosphate [1,2]. It has two functional domains: one for the aldol cleavage of indoleglycerol phosphate to indole andglyceraldehyde 3-phosphate and the other for the synthesis of tryptophan fromindole and serine. In bacteria and plants [3], each domain is found on a separate subunit (alpha and beta chains), while in fungi the two domains are fused together on a single multifunctional protein. A conserved region that contains three conserved acidic residues has been selected as a signature pattern for the alpha chain. The first and the third acidic residues are believed to serve as proton donors/acceptors in the enzyme's catalytic mechanism.

Consensus pattern: [LIVM]-E-[LIVM]-G-x(2)-[FYC]-[ST]-[DE]-[PA]-[LIVMY]- [AGLI]-[DE]-G

[1] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989).[2] Hyde C.C., Miles E.W. Bio/Technology 8:27-32(1990).[3] Berlyn M.B., Last R.L., Fink G.R. Proc. Natl. Acad. Sci. U.S.A. 86:4604-4608(1989).

674. Tryptophan synthase beta chain pyridoxal-phosphate attachment site

Tryptophan synthase catalyzes the last step in the biosynthesis of tryptophan: the conversion of indoleglycerol phosphate and serine, totryptophan and glyceraldehyde 3-phosphate [1,2]. It has two functional domains: one for the aldol cleavage of indoleglycerol phosphate to indole andglyceraldehyde 3-phosphate and the other for the synthesis of tryptophan fromindole and serine. In bacteria and plants [3], each domain is found on a separate subunit (alpha and beta chains), while in fungi the two domains arefused together on a single multifunctional protein. The beta chain of the enzyme requires pyridoxal-phosphate as a cofactor. The pyridoxal-phosphate group is attached to a lysine residue. The region around this lysine residue also contains two histidine residues which are part of the pyridoxal-phosphate binding site. The signature pattern for the tryptophansynthase beta chain is derived from that conserved region.

-Consensus pattern: [LIVM]-x-H-x-G-[STA]-H-K-x-N [K is the pyridoxal-P attachment site]

[1] Crawford I.P. Annu. Rev. Microbiol. 43:567-600(1989).[2] Hyde C.C., Miles E.W. Bio/Technology 8:27-32(1990).[3] Berlyn M.B., Last R.L., Fink G.R. Proc. Natl. Acad. Sci. U.S.A. 86:4604-4608(1989).

675. Serine proteases, trypsin family, active sites

The catalytic activity of the serine proteases from the trypsin family is provided by a charge relay system involving an aspartic acid residue hydrogen-bonded to a histidine, which itself is hydrogen-bonded to a serine. The sequences in the vicinity of the active site serine and histidine residues are well conserved in this family of proteases [1]. A partial list of proteases known to belong to the trypsin family is shown below. - Acrosin. - Blood coagulation factors VII, IX, X, XI and XII, thrombin, plasminogen, and protein C. - Cathepsin G. - Chymotrypsins. - Complement components C1r, C1s, C2, and complement factors B, D and I. - Complement-activating component of RA-reactive factor. - Cytotoxic cell proteases (granzymes A to H). - Duodenase I. - Elastases 1, 2, 3A, 3B (protease E), leukocyte (medullasin). - Enterokinase (EC 3.4.21.9) (enteropeptidase). - Hepatocyte growth factor activator. - Hepsin. - Glandular (tissue) kallikreins (including EGF-binding protein types A, B, and C, NGF-gamma chain, gamma-renin, prostate specific antigen (PSA) and tonin). - Plasma kallikrein. - Mast cell proteases (MCP) 1 (chymase) to 8. - Myeloblastin (proteinase 3) (Wegener's autoantigen). - Plasminogen activators (urokinase-type, and tissue-type). - Trypsins I, II, III, and IV. - Tryptases. - Snake venom proteases such as ancrod, batroxobin, cerastobin, flavoxobin, and protein C activator. - Collagenase from common cattle grub and collagenolytic protease from Atlantic sand fiddler crab. - Apolipoprotein(a). - Blood fluke cercarial protease. - Drosophila trypsin like proteases: alpha, easter, snake-locus. - Drosophila protease stubble (gene sb). - Major mite fecal allergen Der p III. All the above proteins belong to family S1 in the classification of peptidases[2,E1] and originate from eukaryotic species. It should be noted thatbacterial proteases that belong to family S2A are similar enough in the regions of the active site residues that they can be picked up by the same patterns. These proteases are listed below. - Achromobacter lyticus protease I. - Lysobacter alpha-lytic protease. - Streptogrisin A and B (Streptomyces proteases A and B). - Streptomyces griseus glutamyl endopeptidase II. - Streptomyces fradiae proteases 1 and 2. Consensus pattern: [LIVM]-[ST]-A-[STAG]-H-C [H is the active site residue]

Consensus pattern: [DNSTAGC]-[GSTAPIMVQH]-x(2)-G-[DE]-S-G-[GS]-[SAPHV]-[LIVMFYWH]-[LIVMFYSTANQH] [S is the active site residue]

[1] Brenner S. Nature 334:528-530(1988).[2] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).[E1]

676. (tsp) Thrombospondin type 1 domain

[1] Bork P; FEBS lett 1993;327:125-130.

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677. Tubulin subunits alpha, beta, and gamma signature

Tubulins [1,2], the major constituent of microtubules are dimeric proteins which consist of two closely related subunits (alpha and beta). Tubulin binds two molecules of GTP at two different sites (N and E). At the E (Exchangeable) site, GTP is hydrolyzed during incorporation into the microtubule. Near the E site is an invariant region rich in glycines which is found in both chains andwhich is now [3] said to control the access of the nucleotide to its binding site. A signature pattern was developed from this region. With the exception of the simple eukaryotes, most species express a variety of closely related alpha and beta isotypes. In most species there is a third member of the tubulin family: gamma tubulin. Gamma tubulin is found at microtubule organizing centers (MTOC) such as the spindle poles or the centrosome, suggesting that it is involved in the minus-end nucleation of microtubule assembly [4].

Consensus pattern: [SAG]-G-G-T-G-[SA]-G

[1] Cleveland D.W., Sullivan K.F. Annu. Rev. Biochem. 54:331-365(1985).[2] Joshi H.C., Cleveland D.W. Cell Motil. Cytoskeleton 16:159-163(1990).[3] Hesse J., Thierauf M., Ponstingl H. J. Biol. Chem. 262:15472-15475(1987).[4] Joshi H.C. BioEssays 15:637-643(1993).

Tubulin-beta mRNA autoregulation signal

The stability of beta-tubulin mRNAs are autoregulated by their own translation product [1]. Unpolymerized tubulin subunits bind directly (or activate a factor(s) which binds co-translationally) to the nascent N-terminus of beta-tubulin. This binding is transduced through the adjacent ribosomes to activatean RNAse that degrades the polysome-bound mRNA. The recognition element has been shown to be the first four amino acids of beta-tubulin: Met-Arg-Glu-Ile. Mutations to this sequence abolish the autoregulation effect (except for the replacement of Glu by Asp); transposition of this sequence to an internal region of a polypeptide also suppresses the autoregulatory effect.

Consensus pattern: <M-R-[DE]-[IL]

[1] Cleveland D.W. Trends Biochem. Sci. 13:339-343(1988).

678. (tRNA-synt 2c) Aminoacyl-transfer RNA synthetases class-II signatures. Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure. The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7].Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns have been derived from two of these regions.

Consensus pattern: [FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]-

Consensus pattern: [GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]-

[1] Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).[2] Delarue M., Moras D. BioEssays 15:675-687(1993).[3] Schimmel P. Trends Biochem. Sci. 16:1-3(1991).[4] Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991). [5] Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).[6] Cusack S. Biochimie 75:1077-1081(1993).[7] Cusack S., Berthet-Colominas C., Haertlein M., Nassar

N., Leberman R. Nature 347:249-255(1990).[8] Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

679. UBA-domain

The UBA-domain (ubiquitin associated domain) is a novel sequence motif found in several proteins having connections to ubiquitin and the ubiquitination pathway. The structure of the UBA domain consists of a compact three helix bundle [1]. Number of members: 84

[1] Structure of a human DNA repair protein UBA domain that interacts with HIV-1 Vpr. Dieckmann T, Withers-Ward ES, Jarosinski MA, Liu CF, Chen IS, Feigon J; Nat Struct Biol 1998;5:1042-1047.

680. UBX domain

Domain present in ubiquitin-regulatory proteins. Present in FAF1 and Shp1p.Number of members: 19

[1] The UBA domain: a sequence motif present in multiple enzyme classes of the ubiquitination pathway. Hofmann K, Bucher P; Trends Biochem Sci 1996;21:172-173.

681. (UCH) Ubiquitin carboxyl-terminal hydrolases family 1 cysteine active site

Ubiquitin carboxyl-terminal hydrolases (UCH) (deubiquitinating enzymes) [1,2] are thiol proteases that recognize and hydrolyze the peptide bond at the C-terminal glycine of ubiquitin. These enzymes are involved in the processing of poly-ubiquitin precursors as well as that of ubiquinated proteins. There are two distinct families of UCH. The first class consist of enzymes ofabout 25 Kd and is currently represented by: - Mammalian isozymes L1 and L3. - Yeast YUH1. - Drosophila Uch.One of the active site residues of class-I UCH [3] is a cysteine. A signature pattern has been derived from the region around that residue. Consensus pattern: Q-x(3)-N-[SA]-C-G-x(3)-[LIVM](2)-H-[SA]-[LIVM]-[SA] [C is the active site residue

[1] Jentsch S., Seufert W., Hauser H.-P. Biochim. Biophys. Acta 1089:127-139(1991).[2] D'andrea A., Pellman D. Crit. Rev. Biochem. Mol. Biol. 33:337-352(1998).[3] Johnston

S.C., Larsen C.N., Cook W.J., Wilkinson K.D., Hill C.P. EMBO J. 16:3787-3796(1997).[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

682. Ubiquitin carboxyl-terminal hydrolases family 2 signatures (UCH-1)

Ubiquitin carboxyl-terminal hydrolases (UCH) (deubiquitinating enzymes) [1,2] are thiol proteases that recognize and hydrolyze the peptide bond at the C-terminal glycine of ubiquitin. These enzymes are involved in the processing of poly-ubiquitin precursors as well as that of ubiquinated proteins. There are two distinct families of UCH. The second class consist of largeproteins (800 to 2000 residues) and is currently represented by: - Yeast UBP1, UBP2, UBP3, UBP4 (or DOA4/SSV7), UBP5, UBP7, UBP9, UBP10, UBP11, UBP12, UBP13, UBP14, UBP15 and UBP16. - Human tre-2. - Human isopeptidase T. - Human isopeptidase T-3. - Mammalian Ode-1. - Mammalian Unp. - Mouse Dub-1. - Drosophila fat facets protein (gene faf). - Mammalian faf homolog. - Drosophila D-Ubp-64E. - Caenorhabditis elegans hypothetical protein R10E11.3. - Caenorhabditis elegans hypothetical protein K02C4.3.These proteins only share two regions of similarity. The first region containsa conserved cysteine which is probably implicated in the catalytic mechanism. The second region contains two conserved histidines residues, one of which is also probably implicated in the catalytic mechanism. Signature patterns for both conserved regions have been developed.

Consensus pattern: G-[LIVMFY]-x(1,3)-[AGC]-[NASM]-x-C-[FYW]-[LIVMC]-[NST]-[SACV]-x-[LIVMS]-Q [C is the putative active site residue]

Consensus pattern: Y-x-L-x-[SAG]-[LIVMFT]-x(2)-H-x-G-x(4,5)-G-H-Y [The two H's are putative active site residues]

[1] Jentsch S., Seufert W., Hauser H.-P. Biochim. Biophys. Acta 1089:127-139(1991).[2] D'andrea A., Pellman D. Crit. Rev. Biochem. Mol. Biol. 33:337-352(1998).[3] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

683. Ubiquitin carboxyl-terminal hydrolases family 2 signatures (UCH-2)

Ubiquitin carboxyl-terminal hydrolases (UCH) (deubiquitinating enzymes) [1,2] are thiol proteases that recognize and hydrolyze the peptide bond at the C-terminal glycine of ubiquitin. These enzymes are involved in the processing of poly-ubiquitin precursors as well as that of ubiquinated proteins. There are two distinct families of UCH. The second class consist of largeproteins (800 to 2000 residues) and is currently represented by: - Yeast UBP1, UBP2, UBP3, UBP4 (or DOA4/SSV7), UBP5, UBP7, UBP9, UBP10, UBP11, UBP12, UBP13, UBP14, UBP15 and UBP16. - Human tre-2. - Human isopeptidase T. - Human isopeptidase T-3. - Mammalian Ode-1. - Mammalian Unp. - Mouse Dub-1. - Drosophila fat facets protein (gene faf). - Mammalian faf homolog. - Drosophila D-Ubp-64E. - Caenorhabditis elegans hypothetical protein R10E11.3. - Caenorhabditis elegans hypothetical protein K02C4.3.These proteins only share two regions of similarity. The first region containsa conserved cysteine which is probably implicated in the catalytic mechanism. The second region contains two conserved histidines residues, one of which is also probably implicated in the catalytic mechanism. Signature patterns for both conserved regions have been developed.

Consensus pattern: G-[LIVMFY]-x(1,3)-[AGC]-[NASM]-x-C-[FYW]-[LIVMC]-[NST]-[SACV]-x-[LIVMS]-Q [C is the putative active site residue]

Consensus pattern: Y-x-L-x-[SAG]-[LIVMFT]-x(2)-H-x-G-x(4,5)-G-H-Y [The two H's are putative active site residues]

[1] Jentsch S., Seufert W., Hauser H.-P. Biochim. Biophys. Acta 1089:127-139(1991).[2] D'andrea A., Pellman D. Crit. Rev. Biochem. Mol. Biol. 33:337-352(1998).[3] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:461-486(1994).

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684. UDP-glycosyltransferases signature

UDP glycosyltransferases (UGT) are a superfamily of enzymes that catalyzes the addition of the glycosyl group from a UTP-sugar to a small hydrophobic molecule. This family currently consist of: - Mammalian UDP-glucoronosyl transferases (UDPGT) [1,2]. A large family of membrane-bound microsomal enzymes which catalyze the transfer of glucuronic acid to a wide variety of exogenous and endogenous lipophilic substrates. These enzymes are of major importance in the detoxification and subsequent elimination of xenobiotics such as drugs and carcinogens. - A large number of putative UDPGT from Caenorhabditis elegans. - Mammalian 2-hydroxyacylsphingosine 1-beta-galactosyltransferase [3] (also known as UDP-galactose-ceramide galactosyltransferase). This enzyme catalyzes the transfer of galactose to ceramide, a key enzymatic step in the biosynthesis of galactocerebrosides, which are abundant sphingolipids of the myelin membrane of the central nervous system and peripheral nervous system. - Plants flavonol O(3)-glucosyltransferase. An enzyme [4] that catalyzes the transfer of glucose from UDP-glucose to a flavanol. This reaction is essential and one of the last steps in anthocyanin pigment biosynthesis. - Baculoviruses ecdysteroid UDP-glucosyltransferase (EC 2.4.1.-) [5] (egt). This enzyme catalyzes the transfer of glucose from UDP-glucose to ectysteroids which are insect molting hormones. The expression of egt in the insect host interferes with the normal insect development by blocking the molting process. - Prokaryotic zeaxanthin glucosyl transferase (gene crtX), an enzyme involved in carotenoid biosynthesis and that catalyses the glycosylation reaction which converts zeaxanthin to zeaxanthin-beta- diglucoside. - Streptomyces macrolide glycosyltransferases [6]. These enzymes specifically inactivates macrolide anitibiotics via 2'-O-glycosylation using UDP-glucose.These enzymes share a conserved domain of about 50 amino acid residues locatedin their C-terminal section and from which a pattern has been extracted todetect them. Consensus pattern: [FW]-x(2)-Q-x(2)-[LIVMYA]-[LIMV]-x(4,6)-[LVGAC]-[LVFYA]-[LIVMF]-[STAGCM]-[HNQ]-[STAGC]-G-x(2)-[STAG]-x(3)-[STAGL]- [LIVMFA]-x(4)-[PQR]-[LIVMT]-x(3)-[PA]-x(3)-[DES]-[QEHN]

[1] Dutton G.J. (In) Glucoronidation of drugs and other compounds, Dutton G.J., Ed., pp 1-78, CRC Press, Boca Raton, (1980).[2] Burchell B., Nebert D.W., Nelson D.R., Bock K.W., Iyanagi T., Jansen P.L., Lancet D., Mulder G.J., Chowdhury J.R., Siest G., Tephly T.R., Mackenzie P.I. DNA Cell Biol. 10:487-494(1991).[3] Schulte S., Stoffel W. Proc. Natl. Acad. Sci. U.S.A. 90:10265-10269(1993).[4] Furtek D., Schiefelbein J.W., Johnston F., Nelson O.E. Jr. Plant Mol. Biol. 11:473-481(1988).[5] O'Reilly D.R., Miller L.K. Science 245:1110-1112(1989).[6] Hernandez C., Olano C., Mendez C., Salas J.A. Gene 134:139-140(1993).

685. UDP-glucose/GDP-mannose dehydrogenase family

The UDP-glucose/GDP-mannose dehydrogenaseses are a small group of enzymes which possesses the ability to catlyze the NAD-dependent 2-fold oxidation of an alcholol to an acid without the release of an aldehyde intermediate [2]. Number of members: 55

[1] Purification and characterization of guanosine diphospho-D-mannose dehydrogenase. A key enzyme in the biosynthesis of alginate by Pseudomonas aeruginosa. Roychoudhury S, May TB, Gill JF, Singh SK, Feingold DS, Chakrabarty AM; J Biol Chem 1989;264:9380-9385. [2] Properties and kinetic analysis of UDP-glucose dehydrogenase

from group A streptococci. Irreversible inhibition by UDP-chloroacetol. Campbell RE, Sala RF, van de Rijn I, Tanner ME; J Biol Chem 1997;272:3416-3422.

686. Uracil-DNA glycosylase signature

Uracil-DNA glycosylase (EC 3.2.2.-) (UNG) [1] is a DNA repair enzyme that excises uracil residues from DNA by cleaving the N-glycosylic bond. Uracil in DNA can arise as a result of misincorportation of dUMP residues by DNA polymerase or deamination of cytosine. The sequence of uracil-DNA glycosylase is extremely well conserved [2] in bacteria and eukaryotes as well as in herpes viruses. More distantly related uracil-DNA glycosylases are also found in poxviruses [3].In eukaryotic cells, UNG activity is found in both the nucleus and the mitochondria. Human UNG1 protein is transported to both the mitochondria and the nucleus [4]. The N-terminal 77 amino acids of UNG1 seem to be required for mitochondrial localization [4], but the presence of a mitochondrial transitpeptide has not been directly demonstrated. As a signature for this type of enzyme, the most N-termina conserved region has been selected. This region contains an aspartic acid residue which has been proposed, based on X-ray structures [5,6] to act as a general base in the catalytic mechanism. Consensus pattern: [KR]-[LIV]-[LIVC]-[LIVM]-x-G-[QI]-D-P-Y [D is the active site residue]-

[1] Sancar A., Sancar G.B. Annu. Rev. Biochem. 57:29-67(1988).[2] Olsen L.C., Aasland R., Wittwer C.U., Krokan H.E., Helland D.E. EMBO J. 8:3121-3125 (1989).[3] Upton C., Stuart D.T., McFadden G. Proc. Natl. Acad. Sci. U.S.A. 90:4518-4522(1993).[4] Slupphaug G., Markussen F.-H., Olsen L.C., Aasland R., Aarsaether N., Bakke O., Krokan H.E., Helland D.E. Nucleic Acids Res. 21:2579-2584(1993).[5] Savva R., McAuley-Hecht K., Brown T., Pearl L. Nature 373:487-493(1995).[6] Mol C.D., Arvai A.S., Slupphaug G., Kavli B., Alseth I., Krohan H.E., Tainer J.A. Cell 80:869-878(1995).[7] Muller S.J., Caradonna S. Biochim. Biophys. Acta 1088:197-207(1991).[8] Meyer-Siegler K., Mauro D.J., Seal G., Wurzer J., Deriel J.K., Sirover M.A. Proc. Natl. Acad. Sci. U.S.A. 88:8460-8464(1991).[9] Muller S.J., Caradonna S. J. Biol. Chem. 268:1310-1319(1993).[10] Barnes D.E., Lindahl T., Sedgwick B. Curr. Opin. Cell Biol. 5:424-433(1993).

687. Uncharacterized protein family UPF0001 signature

The following uncharacterized proteins have been shown [1] to share regions ofsimilarities: - Yeast chromosome II hypothetical protein YBL036c. - Caenorhabditis elegans hypothetical protein F09E5.8. - Bacillus subtilis hypothetical protein ylmE. - Escherichia coli hypothetical protein yggS and HI0090, the corresponding Haemophilus influenzae protein. - Helicobacter pylori hypothetical protein HP0395. - Mycobacterium tuberculosis hypothetical protein MtCY270.20. - Synechocystis strain PCC 6803 hypothetical protein slr0556. - A Pseudomonas aeruginosa hypothetical protein in pilT 5'region. - A Vibrio alginolyticus hypothetical protein in pilT 5'region. These are proteins of from 25 to 30 Kd which contain a number of conserved regions. The best conserved region which is located in the first third of these proteins has been selected as a signature pattern.

Consensus pattern: [FW]-H-[FM]-[IV]-G-x-[LIV]-Q-x-[NKR]-K-x(3)-[LIV]

[1] Bairoch A., Rudd K.E. Unpublished observations (1996).

09555327.061700

688. Uncharacterized protein family UPF0003 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli protein aefA. - Escherichia coli hypothetical protein yggB. - Escherichia coli hypothetical protein yjeP and HI0195.1, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein ynaI. - Bacillus subtilis hypothetical protein yhdY. - Helicobacter pylori hypothetical protein HP0415. - Synechocystis strain PCC 6803 hypothetical protein slr0639. - Archaeoglobus fulgidus hypothetical protein AF1546. - Methanococcus jannaschii hypothetical protein MJ0170. - Methanococcus jannaschii hypothetical protein MJ1143.The size of these proteins range from 30 to 120 Kd. They all contain a number of transmembrane regions. The best conserved region which is located in and just after the last potential transmembrane region has been selected as a signature pattern,.

Consensus pattern: G-[STIF]-V-x(2)-[LIVM]-x(6)-[LIVMF]-x(3)-[DQ]-x(3)-[LIV]- x-[LIV]-P-N-x(2)-[LIVMF]-[LIVFSTA]-x(5)-N

[1] Bairoch A. Unpublished observations (1997).

689. Uncharacterized protein family UPF0004 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yliG. - Escherichia coli hypothetical protein yleA and HI0019, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yqeV. - Helicobacter pylori hypothetical protein HP0269. - Helicobacter pylori hypothetical protein HP0285. - Mycoplasma iowae hypothetical protein in 16S RNA 5'region. - Mycobacterium leprae hypothetical protein B2235_C2_195. - Pseudomonas aeruginosa hypothetical protein in hemL 3'region. - Synechocystis strain PCC 6803 hypothetical protein slr0082. - Synechocystis strain PCC 6803 hypothetical protein sll0996. - Methanococcus jannaschii hypothetical protein MJ0865. - Methanococcus jannaschii hypothetical protein MJ0867. - Caenorhabditis elegans hypothetical protein F25B5.5.The size of these proteins range from 47 to 61 Kd. They contain six conserved cysteines, three of which are clustered in a region that can be used as asignature pattern.

Consensus pattern: [LIVM]-x-[LIVMT]-x(2)-G-C-x(3)-C-[STAN]-[FY]-C-x-[LIVM]- x(4)-G

[1] Bairoch A. Unpublished observations (1997).

690. Uncharacterized protein family UPF0005 signature

The following proteins seems to be evolutionary related [1]: - Mammalian protein TEGT (Testis Enhanced Gene Transcript). - Escherichia coli hypothetical protein yccA and HI0044, the corresponding Haemophilus influenzae protein. - A probable Pseudomonas aeruginosa ortholog of yccA. These are proteins of about 25 Kd which seem to contain seven transmembranedomains. A signature pattern that corresponds to a region that starts with the beginning of the third transmembrane domain and ends in the middle of the fourth one has been developed.

Consensus pattern: G-[LIVM](2)-[SA]-x(5,8)-G-x(2)-[LIVM]-G-P-x-L-x(4)-[SAG]- x(4,6)-[LIVM](2)-x(2)-A-x(3)-T-A-[LIVM](2)-F

[1] Walter L., Marynen P., Szpirer J., Levan G., Guenther E. Genomics 28:301-304(1995).

691. Uncharacterized protein family UPF0006 signatures

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast chromosome II hypothetical protein YBL055c. - Escherichia coli hypothetical protein ycfH and HI0454, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yigW. - Escherichia coli hypothetical protein yjjV and HI0081, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yabD. - Haemophilus influenzae hypothetical protein HI1664. - Mycoplasma genitalium hypothetical protein MG009. These are proteins of from 24 to 47 Kd which contain a number of conserved regions. They can be picked up in the database by the following patterns.

Consensus pattern: [LIVMFY](2)-D-[STA]-H-x-H-[LIVMF]-[DN

Consensus pattern: P-[LIVM]-x-[LIVM]-H-x-R-x-[TA]-x-[DE

Consensus pattern: [LVSA]-[LIVA]-x(2)-[LIVM]-[PS]-x(3)-L-[LIVM]-[LIVMS]-E-T- D-x-P

[1] Bairoch A., Rudd K.E. Unpublished observations (1995).

692. Uncharacterized protein family UPF0007 signature

The following proteins seems to be evolutionary related [1]: - Escherichia coli hypothetical protein ygbP and HI0672, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yacM. - Mycobacterium tuberculosis hypothetical protein MtCY06G11.29c. - Synechocystis strain PCC 6803 hypothetical protein slr0951. - A Rhodobacter capsulatus hypothetical protein in nifR3 5'region. Except for the Rhodobacter protein which contains a C-terminal extension, all these proteins have from 225 to 236 amino acids. They are hydrophilic proteins that can be picked up in the database by the following pattern.

Consensus pattern: V-L-[IV]-H-D-[GA]-A-R

[1] Bairoch A. Unpublished observations (1997).

693. Uncharacterized protein family UPF0015 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast chromosome II hypothetical protein YBR002c. - Yeast chromosome XIII hypothetical protein YMR101c. - Escherichia coli hypothetical protein yaeU and HI0920, the corresponding Haemophilus influenzae protein. - Helicobacter pylori hypothetical protein HP1221. - Mycobacterium leprae hypothetical protein B1937_F2_65. - A Corynebacterium glutamicum hypothetical protein in aroF 3'region. - A Streptomyces fradiae hypothetical protein in transposon Tn4556. - Synechocystis strain PCC 6803 hypothetical protein sll0505. - Methanococcus jannaschii hypothetical protein MJ1372.These are proteins of about 26 to 40 Kd whose central region is well conserved. They can be picked up in the database by the following pattern.

Consensus pattern: [DE]-[LIVMF](3)-R-T-[SG]-G-x(2)-R-x-S-x-[FY]-[LIVM](2)-W-Q-

[1] Wolfe K.H., Lohan A.J.E. Yeast 10:S41-S46(1994).

694. Uncharacterized protein family UPF0016 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast hypothetical protein YBR187w. - Fission yeast hypothetical protein SpAC17G8.08c. - Mouse protein pFT27. - Synechocystis strain PCC 6803 hypothetical protein sll0615. These are hydrophobic proteins of 200 to 320 amino acids that seem to contain six or seven transmembrane domains. A conserved region which seems, in the eukaryotic proteins of this family, to directly follow the second transmembrane domain has been selected as a signature pattern.

Consensus pattern: E-[LIVM]-G-D-K-T-F-[LIVMF](2)-A-

[1] Bairoch A. Unpublished observations (1996).

695. Uncharacterized protein family UPF0021 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast chromosome VII hypothetical protein YGL211w. - Dictyostelium discoideum protein veg136. - Methanococcus jannaschii hypothetical proteins MJ1157 and MJ1478.These are proteins of from 300 to 36o residues. They can be picked up in thedatabase by the following pattern which is located in their N-terminalsection.

Consensus pattern: C-K-x(2)-F-x(4)-E-x(22,23)-S-G-G-K-D

[1] Bairoch A. Unpublished observations (1997).

696. Uncharacterized protein family UPF0023 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Mouse protein 22A3. - Yeast chromosome XII hypothetical protein YLR022c. -

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Caenorhabditis elegans hypothetical protein W06E11.4. - Methanococcus jannaschii hypothetical protein MJ0592.These are hydrophilic proteins of about 30 Kd. They can be picked up in the database by the following pattern.

Consensus pattern: D-x-D-E-[LIV]-L-x(4)-V-F-x(3)-S-K-G-

[1] Bairoch A. Unpublished observations (1997).

697. Uncharacterized protein family UPF0024 signature. The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein ygbO and HI0701, the corresponding Haemophilus influenzae protein. - Helicobacter pylori hypothetical protein HP0926. - Yeast chromosome XV hypothetical protein YOR243c. - Caenorhabditis elegans hypothetical protein B0024.11. - Methanococcus jannaschii hypothetical proteins MJ0588 and MJ1364.These are hydrophilic proteins of from 39 to 77 Kd. They can be picked up in the database by the following pattern.

Consensus pattern: G-x-K-D-[KR]-x-A-[LV]-T-x-Q-x-[LIVF]-[SGC]-

[1] Bairoch A. Unpublished observations (1997).

09530526 .061600

698. Uncharacterized protein family UPF0025 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yfcE. - Bacillus subtilis hypothetical protein ysnB. - Mycoplasma genitalium and pneumoniae hypothetical protein MG207. - Methanococcus jannaschii hypothetical proteins MJ0623 and MJ0936. These are hydrophilic proteins of about 20 Kd. They can be picked up in thedatabase by the following pattern.

Consensus pattern: D-V-[LIV]-x(2)-G-H-[ST]-H-x(12)-[LIVMF]-N-P-G

[1] Bairoch A. Unpublished observations (1997).

699. Uncharacterized protein family UPF0029 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Yeast chromosome III hypothetical protein YCR59c. - Yeast chromosome IV hypothetical protein YDL177C. - Escherichia coli hypothetical protein yigZ and HI0722, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yvyE. - A Thermus aquaticus hypothetical protein in pol 5'region. These proteins can be picked up in the database by the following pattern.

Consensus pattern: G-x(2)-[LIVM](2)-x(2)-[LIVM]-x(4)-[LIVM]-x(5)-[LIVM](2)-x- R-[FYW](2)-G-G-x(2)-[LIVM]-G

[1] Koonin E.V., Bork P., Sander C. EMBO J. 13:493-503(1994).

700. Uncharacterized protein family UPF0030 signature

The following uncharacterized proteins have been shown [1] to be highly similar: - Yeast chromosome VI hypothetical protein YFL060c. - Yeast chromosome XIII hypothetical protein YMR095c. - Yeast chromosome XIV hypothetical protein YNL334c. - Bacillus subtilis hypothetical protein yaaE. - Haemophilus influenzae hypothetical protein HI1648. - Methanococcus jannaschii hypothetical protein MJ1661.These are hydrophilic proteins of about 19 to 25 Kd. They can be picked up inthe database by the following pattern.

Consensus pattern: [GA]-L-I-[LIV]-P-G-G-E-S-T-[STA]

[1] Bairoch A. Unpublished observations (1997).

09535360.061502

701. Uncharacterized protein family UPF0032 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yigU and HI0188, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein ycbT. - Mycobacterium tuberculosis hypothetical protein MtCY49.33c and U2126A, the corresponding Mycobacterium leprae protein. - Synechocystis strain PCC 6803 hypothetical protein sll0194. - Odontella sinensis and Porphyra purpurea chlroplast hypothetical protein ycf43.These proteins have from 245 to 317 amino acids and seem to contain at least six or seven transmembrane regions. A conserved region located in the central section of these proteins has been developed as a signature pattern,.

Consensus pattern: Y-x(2)-F-[LIVMA](2)-x-L-x(4)-G-x(2)-F-[EQ]-[LIVMF]-P- [LIVM] –

[1] Bairoch A., Rudd K.E. Unpublished observations (1996).

702. Uncharacterized protein family UPF0034 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yhdG and HI0979, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yjbN and HI0634, the corresponding Haemophilus influenzae protein. - Escherichia coli hypothetical protein yohI and HI0270, the corresponding Haemophilus influenzae protein. - Bacillus subtilis hypothetical protein yacF. - Rhodobacter capsulatus protein nifR3 and related proteins in Azospirillum brasilense and Rhizobium leguminosarum. - Synechocystis strain PCC 6803 hypothetical protein slr0644. - Synechocystis strain PCC 6803 hypothetical protein sll0926. - Caenorhabditis elegans hypothetical protein C45G9.2. - Yeast protein SMM1. - Yeast hypothetical protein YLR401c. - Yeast hypothetical protein YLR405w. - Yeast hypothetical protein YML080w. Although it has been proposed [2] that Rhodobacter capsulatus nifR3 is a transcriptional regulatory protein, it is believed that these proteins constitute a family of enzymes whose active site could include a conserved cysteine which has been used as the central part of a signature pattern.

Consensus pattern: [LIVM]-[DNG]-[LIVM]-N-x-G-C-P-x(3)-[LIVMASQ]-x(5)-G-[SAC]

[1] Bairoch A., Rudd K.E. Unpublished observations (1995).[2] Foster-Hartnett D., Cullen P.J., Gabbert K.K., Kranz R.G. Mol. Microbiol. 8:903-914(1993).

703. Uncharacterized protein family UPF0038 signature

The following uncharacterized proteins have been shown [1] to share regions of similarities: - Escherichia coli hypothetical protein yacE and HI0890, the corresponding Haemophilus influenzae protein. - Mycobacterium tuberculosis hypothetical protein MtCY01B2.23 and O410, the corresponding Mycobacterium leprae protein. - Synechocystis strain PCC 6803 hypothetical protein slr0553. - Other hypothetical proteins from Aeromonas hydrophila, Bacteroides nodosus, Neisseria gonorrhoeae, Pseudomonas putida, Thermus thermophilus and Xanthomonas campestris. - Human hypothetical protein pOV-2. - Yeast hypothetical protein YDR196C. - Caenorhabditis elegans hypothetical protein T05G5.5.These proteins all contain, in their N-terminal extremity, an ATP/GTP-binding motif 'A' (P-loop) (see <PDOC00017>). The size of these proteins range from 200 to 290 residues (with the exception of the Mycobacterial sequences which are are 410 residues long). A conseved region some 50 residues away from the ATP-binding P-loop has been developed as a signature pattern.

Consensus pattern: G-x-[LI]-x-R-x(2)-L-x(4)-F-x(8)-[LIV]-x(5)-P-x-[LIV] –

[1] Rudd K.E., Bairoch A. Unpublished observations (1997).

704. Ubiquitin-conjugating enzymes active site

Ubiquitin-conjugating enzymes (UBC or E2 enzymes) [1,2,3] catalyze the covalent attachment of ubiquitin to target proteins. An activatedubiquitin moiety is transferred from an ubiquitin-activating enzyme (E1) to E2which later ligates ubiquitin directly to substrate proteins with or without the assistance of 'N-end' recognizing proteins (E3). In most species there are many forms of UBC (at least 9 in yeast) which are implicated in diverse cellular functions. A cysteine residue is required for ubiquitin-thiolester formation. There is a single conserved cysteine in UBC's and the region around that residue isconserved in the sequence of known UBC isozymes. That region has been used as a signature pattern.

Consensus pattern: [FYWLSP]-H-[PC]-[NH]-[LIV]-x(3,4)-G-x-[LIV]-C-[LIV]-x- [LIV] [C is the active site residue]

[1] Jentsch S., Seufert W., Sommer T., Reins H.-A. Trends Biochem. Sci. 15:195-198(1990).[2] Jentsch S., Seufert W., Hauser H.-P. Biochim. Biophys. Acta 1089:127-139(1991).[3] Hershko A. Trends Biochem. Sci. 16:265-268(1991).

705. Uroporphyrinogen decarboxylase signatures

Uroporphyrinogen decarboxylase (URO-D), the fifth enzyme of the heme biosynthetic pathway, catalyzes the sequential decarboxylation of the four acetyl side chains of uroporphyrinogen to yield coproporphyrinogen [1].URO-D deficiency is responsible for the Human genetic diseases familialporphyria cutanea tarda (fPCT) and hepatoerythropoietic porphyria (HEP).The sequence of URO-D has been well conserved throughout evolution. The best conserved region is located in the N-terminal section; it contains a perfectlyconserved hexapeptide. There are two arginine residues in this hexapeptide which could be involved in the binding, via salt bridges, to the carboxylgroups of the propionate side chains of the substrate. This region has been used as a signature pattern. A second signature pattern is based on a another well conserved region which is located in the central section of the protein.

Consensus pattern: P-x-W-x-M-R-Q-A-G-R

Consensus pattern: G-F-[STAGCV]-[STAGC]-x-P-[FYW]-T-[LV]-x(2)-Y-x(2)-[AE]- [GK]

[1] Garey J.R., Labbe-Bois R., Chelstowska A., Rytka J., Harrison L., Kushner J., Labbe P. Eur. J. Biochem. 205:1011-1016(1992).

706. ubiE/COQ5 methyltransferase family signatures

The following methyltransferases have been shown [1] to share regions of similarities: - Escherichia coli ubiE, which is involved in both ubiquinone and menaquinone biosynthesis and which catalyzes the S-adenosylmethionine dependent methylation of 2-polyprenyl-6-methoxy-1,4-benzoquinol into 2-polyprenyl-3- methyl-6-methoxy-1,4-benzoquinol and of demethylmenaquinol into menaquinol. - Yeast COQ5, a ubiquinone biosynthesis methlytransferase. - Bacillus subtilis spore germination protein C2 (gene: gercB or gerC2), a probable menaquinone biosynthesis methlytransferase. - Lactococcus lactis gerC2 homolog. - Caenorhabditis elegans hypothetical protein ZK652.9. - Leishmania donovani amastigote-specific protein A41.These are hydrophilic proteins of about 30 Kd (except for ZK652.9 which is 65Kd). They can be picked up in the database by the following patterns.

Consensus pattern: Y-D-x-M-N-x(2)-[LIVM]-S-x(3)-H-x(2)-W

Consensus pattern: R-V-[LIVM]-K-[PV]-G-G-x-[LIVMF]-x(2)-[LIVM]-E-x-S

[1] Lee P.T., Hsu A.Y., Ha H.T., Clarke C.F. J. Bacteriol. 179:1748-1754(1997).

707. Uricase signature

Uricase (urate oxidase) [1] is the peroxisomal enzyme responsible for the degradation of urate into allantoin. Some species, like primates and birds, have lost the gene for uricase and are therefore unable to degradeurate. Uricase is a protein of 300 to 400 amino acids. A highly conserved region located in the central part of the sequence has been used as a signature pattern.

Consensus pattern: [LV]-x-[LV]-[LIV]-K-[STV]-[ST]-x-[SN]-x-F-x(2)-[FY]-x(4)- [FY]-x(2)-L-x(5)-R

[1] Motojima K., Kanaya S., Goto S. J. Biol. Chem. 263:16677-16681(1988).

708. Universal stress protein family (Usp)

By a wide range of stress conditions members of the Usp family are predicted to be related to the MADS-box proteins transcript_fact and bind to DNA [2]. Number of members: 39

[1] Expression and role of the universal stress protein, UspA, of Escherichia coli during growth arrest. Nystrom T, Neidhardt FC; Mol Microbiol 1994; 11:537-544.
[2] Sequence analysis of eukaryotic developmental proteins: ancient and novel domains. Mushegian AR, Koonin EV; Genetics 1996; 144:817-828.

709. Ubiquitin domain signature and profile

Ubiquitin [1,2,3] is a protein of seventy six amino acid residues, found in all eukaryotic cells and whose sequence is extremely well conserved from protozoan to vertebrates. It plays a key role in a variety of cellular processes, such as ATP-dependent selective degradation of cellular proteins,maintenance of chromatin structure, regulation of gene expression, stress response and ribosome biogenesis. In most species, there are many genes coding for ubiquitin. However they can be classified into two classes. The first class produces polyubiquitin molecules consisting of exact head to tail repeats of ubiquitin. The number of repeats is variable (up to twelve in a Xenopus gene). In the majority of polyubiquitin precursors, there is a final amino-acid after the last repeat. The second class of genes produces precursor proteins consisting of a single copy of ubiquitin fused to a C-terminal extension protein (CEP). There are two types of CEP proteins and both seem to be ribosomal proteins. Ubiquitin is a globular protein, the last four C-terminal residues (Leu-Arg- Gly-Gly) extending from the compact structure to form a 'tail', important for its function. The latter is mediated by the covalent conjugation of ubiquitin to target proteins, by an isopeptide linkage between the C-terminal glycine and the epsilon amino group of lysine residues in the target proteins. There are a number of proteins which are evolutionary related to ubiquitin: - Ubiquitin-like proteins from baculoviruses as well as in some strains of bovine viral diarrhea viruses (BVDV). These proteins are highly similar to their eukaryotic counterparts. - Mammalian protein GDX [4]. GDX is composed of two domains, a N-terminal ubiquitin-like domain of 74 residues and a C-terminal domain of 83 residues with some similarity with the thyroglobulin hormonogenic site. - Mammalian protein FAU [5]. FAU is a fusion protein which consist of a N-terminal ubiquitin-like protein of 74 residues fused to ribosomal protein S30. - Mouse protein NEDD-8 [6], a ubiquitin-like protein of 81 residues. - Human protein BAT3, a large fusion protein of 1132 residues that contains a N-terminal ubiquitin-like domain. - Caenorhabditis elegans protein ubl-1 [7]. Ubl-1 is a fusion protein which consist of a N-terminal ubiquitin-like protein of 70 residues fused to ribosomal protein S27A. - Yeast DNA repair protein RAD23 [8]. RAD23 contains a N-terminal domain that seems to be distantly, yet significantly, related to ubiquitin. - Mammalian RAD23-related proteins RAD23A and RAD23B. - Mammalian BCL-2 binding athanogene-1 (BAG-1). BAG-1 is a protein of 274 residues that contains a central ubiquitin-like domain. - Human spliceosome associated protein 114 (SAP 114 or SF3A120). - Yeast protein DSK2, a protein involved in spindle pole body duplication and which contains a N-terminal ubiquitin-like domain. - Human protein CKAP1/TFCB, Schizosaccharomyces pombe protein alp11 and Caenorhabditis elegans hypothetical protein F53F4.3. These proteins contain a N-terminal ubiquitin domain and a C-terminal CAP-Gly domain. - Schizosaccharomyces pombe hypothetical protein SpAC26A3.16. This protein contains a N-terminal ubiquitin domain. - Yeast protein SMT3. - Human ubiquitin-like proteins SMT3A and SMT3B. - Human ubiquitin-like protein SMT3C (also known as PIC1; Ubl1, Sumo-1; Gmp-1 or Sentrin). This protein is involved in targeting ranGAP1 to the nuclear pore complex protein ranBP2. - SMT3-like proteins in plants and Caenorhabditis elegans. To identify ubiquitin and related proteins, a pattern has been developed based on conserved positions in the central section of the sequence. A profile was also developed that spans the complete length of the ubiquitin domain.

Consensus pattern: K-x(2)-[LIVM]-x-[DESAK]-x(3)-[LIVM]-[PA]-x(3)-Q-x-[LIVM]-[LIVMC]-[LIVMFY]-x-G-x(4)-[DE]

[1] Jentsch S., Seufert W., Hauser H.-P. Biochim. Biophys. Acta 1089:127-139(1991).[2] Monia B.P., Ecker D.J., Croke S.T. Bio/Technology 8:209-215(1990).[3] Finley D., Varshavsky A. Trends Biochem. Sci. 10:343-347(1985).[4] Filippi M., Tribioli C., Toniolo D. Genomics 7:453-457(1990).[5] Olvera J., Wool I.G. J. Biol. Chem. 268:17967-17974(1993).[6] Kumar S., Yoshida Y., Noda M. Biochem. Biophys. Res. Commun. 195:393-399(1993).[7] Jones D., Candido E.P. J. Biol. Chem. 268:19545-19551(1993).[8] Melnick L., Sherman F. J. Mol. Biol. 233:372-388(1993).

710. VHS domain

Domain present in VPS-27, Hrs and STAM. Number of members: 27

711. Vinculin family signatures

Vinculin [1] is a eukaryotic protein that seems to be involved in the attachment of the actin-based microfilaments to the plasma membrane. Vinculinis located at the cytoplasmic side of focal contacts or adhesion plaques. In addition to actin, vinculin interacts with other structural proteins such as talin and alpha-actinins. Vinculin is a large protein of 116 Kd (about a 1000 residues). Structurally the protein consists of an acidic N-terminal domain of about 90 Kd separated from a basic C-terminal domain of about 25 Kd by a proline-rich region of about 50 residues. The central part of the N-terminal domain consists of avariable number (3 in vertebrates, 2 in Caenorhabditis elegans) of repeats of a 110 amino acids domain. Catenins [2] are proteins that associate with the cytoplasmic domain of avariety of cadherins. The association of catenins to cadherins produces a complex which is linked to the actin filament network, and which seems to be of primary importance for cadherins cell-adhesion properties. Three different types of catenins seem to exist: alpha, beta, and gamma. Alpha-catenins are proteins of about 100 Kd which are evolutionary related to vinculin. Interm of their structure the most significant differences are the absence, inalpha-catenin, of the repeated domain and of the proline-rich segment. Two signature patterns for this family of proteins have been devolped. The first pattern is located in the N-terminal section of both vinculin and alpha-catenins and is part, in vinculin, of a domain that seems to be involved with the interaction with talin. The second pattern is based on a conserved regionin the N-terminal part of the repeated domain of vinculin.

Consensus pattern: [KR]-x-[LIVMF]-x(3)-[LIVMA]-x(2)-[LIVM]-x(6)-R-Q-Q-E-L

Consensus pattern: [LIVM]-x-[QA]-A-x(2)-W-[IL]-x-[DN]-P

[1] Otto J.J. Cell Motil. Cytoskeleton 16:1-6(1990).[2] Herrenknecht K., Ozawa M., Eckerskorn C., Lottspeich F., Lenter M., Kemler R. Proc. Natl. Acad. Sci. U.S.A. 88:9156-9160(1991).

712. (Vitellogenin N) Lipoprotein amino terminal region

This family contains regions from: Vitellogenin, Microsomal triglyceride transfer protein and apolipoprotein B-100. These proteins are all involved in lipid transport [1]. This family contains the LV1n chain from lipovitellin, that contains two structural domains.

Number of members: 33

[1] The structural basis of lipid interactions in lipovitellin, a soluble lipoprotein. Anderson TA, Levitt DG, Banaszak LJ Structure 1998;6:895-909.

713. (VMSA) Major surface antigen from hepadnavirus

714. ssDNA binding protein (Viral DNA bp)

This protein is found in herpesviruses and is needed for replication.

715. (Votage CLC) Voltage gated chloride channels

This family of ion channels contains 10 or 12 transmembrane helices. Each protein forms a single pore. It has been shown that some members of this family form homodimers. These proteins contain two CBS domains.

[1] Schmidt-Rose T, Jentsch TJ; J Biol Chem 1997;272:20515-20521.

[2] Zhang J, George AL Jr, Griggs RC, Fouad GT, Roberts J, Kwiecinski H, Connolly AM, Ptacek LJ; Neurology 1996;47:993-998.

716. von Willebrand factor type A domain (vwa)

More von Willebrand factor type A domains? Sequence similarities with malaria thrombospondin-related anonymous protein, dihydropyridine-sensitive calcium channel and inter-alpha-trypsin inhibitor.

09555326.051502

Bork P, Rohde K;
Biochem J 1991;279:908-911.

1. RUGGERI, Z.M. and WARE, J.
von Willebrand factor.
FASEB J. 7 308-316 (1993).

2. COLOMBATTI, A., BONALDO, P. and DOLIANA, R.
Type A modules: interacting domains found in several non-fibrillar collagens and in other extracellular matrix proteins.
MATRIX 13 297-306 (1993).

3. PERKINS, S.J., SMITH, K.F., WILLIAMS, S.C., HARIS, P.I., CHAPMAN, D. and SIM, R.B.
The secondary structure of the von Willebrand factor type A domain in factor B of human complement by Fourier transform infrared spectroscopy. Its occurrence in collagen types VI, VII, XII and XIV, the integrins and other proteins by averaged structure predictions.
J.MOL.BIOL. 238 104-119 (1994).

4. BORK, P. and ROHDE, K.
More von Willebrand factor type A domains? Sequence similarities with malaria thrombospondin-related anonymous protein, dihydropyridine-sensitive calcium channel and inter-alpha-trypsin inhibitor.
BIOCHEM.J. 279 908-910 (1991).

5. EDWARDS, Y.J.K. and PERKINS, S.J.
The protein fold of the von Willebrand factor type A domain is predicted to be similar to the open twisted beta-sheet flanked by alpha-helices found in human ras-p21.
FEBS LETT. 358 283-286 (1995).

6. LEE, J.O., RIEU, P., ARNAOUT, M.A. and LIDDINGTON, R.

Crystal structure of the A domain from the alpha subunit of integrin CR3 (CD11b/CD18).
CELL 80 631-638 (1995).

7. QU, A. and LEAHY, D.J.
Crystal structure of the I-domain from the CD11a/CD18 (LFA-1, alpha L beta 2) integrin.
PROC.NATL.ACAD.SCI.USA 92 10277-10281 (1995).

The von Willebrand factor is a large multimeric glycoprotein found in blood plasma. Mutant forms are involved in the aetiology of bleeding disorders [1]. In von Willebrand factor, the type A domain (vWF) is the prototype for a protein superfamily. The vWF domain is found in various plasma proteins: complement factors B, C2, CR3 and CR4; the integrins (I-domains); collagen types VI, VII, XII and XIV; and other extracellular proteins [2-4]. Proteins that incorporate vWF domains participate in numerous biological events (e.g., cell adhesion, migration, homing, pattern formation, and signal transduction), involving interaction with a large array of ligands [2]. Secondary structure prediction from 75 aligned vWF sequences has revealed a largely alternating sequence of alpha-helices and beta-strands [3]. Fold recognition algorithms were used to score sequence compatibility with a library of known structures: the vWF domain fold was predicted to be a doubly-wound, open, twisted beta-sheet flanked by alpha-helices [5]. 3D structures have been determined for the I-domains of integrins CD11b (with bound magnesium) [6] and CD11a (with bound manganese) [7]. The domain adopts a classic alpha/beta Rossmann fold and contains an unusual metal ion coordination site at its surface. It has been suggested that this site represents a general metal ion-dependent adhesion site (MIDAS) for binding protein ligands [6]. The residues constituting the MIDAS motif in the CD11b and CD11a I-domains are completely conserved, but the manner in which the metal ion is coordinated differs slightly [7].

VWFADOMAIN is a 3-element fingerprint that provides a signature for the vWF domain superfamily. The fingerprint was derived from an initial alignment of 14 sequences. Motif 1 includes the first beta-strand and 3 conserved residues involved in metal ion coordination in I-domains (Asp and 2 serines in positions 8, 10 and 12, respectively); motif 2 spans strands beta-2 and beta-2'; and motif 3 encodes beta-strand 3 and a conserved Asp (in position 7), which coordinates the metal ion [6,7]. Three iterations on OWL27.0 were required to reach convergence, at which point a true set comprising 56 sequences was identified. Numerous partial matches were also found.

717. (WD40) WD domain, G-beta repeat
The ancient regulatory-protein family of WD-repeat proteins.
Neer EJ, Schmidt CJ, Nambudripad R, Smith TF;
Nature 1994;371:297-300.

09535376.061502

Beta-transducin (G-beta) is one of the three subunits (alpha, beta, and gamma) of the guanine nucleotide-binding proteins (G proteins) which act as intermediaries in the transduction of signals generated by transmembrane receptors [1]. The alpha subunit binds to and hydrolyzes GTP; the functions of the beta and gamma subunits are less clear but they seem to be required for the replacement of GDP by GTP as well as for membrane anchoring and receptor recognition.

In higher eukaryotes G-beta exists as a small multigene family of highly conserved proteins of about 340 amino acid residues. Structurally G-beta consists of eight tandem repeats of about 40 residues, each containing a central Trp-Asp motif (this type of repeat is sometimes called a WD-40 repeat). Such a repetitive segment has been shown [E1,2,3,4,5] to exist in a number of other proteins listed below:

- Yeast STE4, a component of the pheromone response pathway. STE4 is a G-beta like protein that associates with GPA1 (G-alpha) and STE18 (G-gamma).
- Yeast MSI1, a negative regulator of RAS-mediated cAMP synthesis. MSI1 is most probably also a G-beta protein.

- Human and chicken protein 12.3. The function of this protein is not known, but on the basis of its similarity to G-beta proteins, it may also function in signal transduction.
- Chlamydomonas reinhardtii gblp. This protein is most probably the homolog of vertebrate protein 12.3.
- Human LIS1, a neuronal protein involved in type-1 lissencephaly [E2].
- Mammalian coatomer beta' subunit (beta'-COP), a component of a cytosolic protein complex that reversibly associates with Golgi membranes to form vesicles that mediate biosynthetic protein transport.

- Yeast CDC4, essential for initiation of DNA replication and separation of the spindle pole bodies to form the poles of the mitotic spindle.
- Yeast CDC20, a protein required for two microtubule-dependent processes: nuclear movements prior to anaphase and chromosome separation.
- Yeast MAK11, essential for cell growth and for the replication of M1 double-stranded RNA.
- Yeast PRP4, a component of the U4/U6 small nuclear ribonucleoprotein with a probable role in mRNA splicing.
- Yeast PWP1, a protein of unknown function.
- Yeast SKI8, a protein essential for controlling the propagation of double-stranded RNA.
- Yeast SOF1, a protein required for ribosomal RNA processing which associates with U3 small nucleolar RNA.
- Yeast TUP1 (also known as AER2 or SFL2 or CYC9), a protein which has been implicated in dTMP uptake, catabolite repression, mating sterility, and many other phenotypes.
- Yeast YCR57c, an ORF of unknown function from chromosome III.
- Yeast YCR72c, an ORF of unknown function from chromosome III.

- Slime mold coronin, an actin-binding protein.
- Slime mold AAC3, a developmentally regulated protein of unknown function.

- Drosophila protein Groucho (formerly known as E(spl); 'enhancer of split'), a protein involved in neurogenesis and that seems to interact with the Notch and Delta proteins.
- Drosophila TAF-II-80, a protein that is tightly associated with TFIID.

The number of repeats in the above proteins varies between 5 (PRP4, TUP1, and Groucho) and 8 (G-beta, STE4, MSI1, AAC3, CDC4, PWP1, etc.). In G-beta and G-beta like proteins, the repeats span the entire length of the sequence, while in other proteins, they make up the N-terminal, the central or the C-terminal section.

A signature pattern can be developed from the central core of the domain (positions 9 to 23).

-Consensus pattern: [LIVMSTAC]-[LIVMFYWSTAGC]-[LIMSTAG]-[LIVMSTAGC]-x(2)-[DN]-
x(2)-[LIVMWSTAC]-x-[LIVMFSTAG]-W-[DEN]-[LIVMFSTAGCN]

[1] Gilman A.G.
Annu. Rev. Biochem. 56:615-649(1987).
[2] Duronio R.J., Gordon J.I., Boguski M.S.
Proteins 13:41-56(1992).
[3] van der Voorn L., Ploegh H.L.
FEBS Lett. 307:131-134(1992).
[4] Neer E.J., Schmidt C.J., Nambudripad R., Smith T.F.
Nature 371:297-300(1994).
[5] Smith T.F., Gaiatzes C.G., Saxena K., Neer E.J.
Biochemistry In Press(1998).

718. WHEP-TRS domain containing proteins

A conserved domain of 46 amino acids has been shown [1] to exist in a number of higher eukaryote aminoacyl-transfer RNA synthetases. This domain is present one to six times in the following enzymes:

- Mammalian multifunctional aminoacyl-tRNA synthetase. The domain is present three times in a region that separates the N-terminal glutamyl-tRNA synthetase domain from the C-terminal prolyl-tRNA synthetase domain.
- Drosophila multifunctional aminoacyl-tRNA synthetase. The domain is present six times in the intercatalytic region.
- Mammalian tryptophanyl-tRNA synthetase. The domain is found at the N-terminal extremity.
- Mammalian, insect, nematode and plant glycyl-tRNA synthetase. The domain is found at the N-terminal extremity [2].
- Mammalian histidyl-tRNA synthetase. The domain is found at the N-terminal extremity.

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This domain, which is called WHEP-TRS, could contain a central alpha-helical region and may play a role in the association of tRNA-synthetases into multienzyme complexes.

A signature pattern based on the first 29 positions of the WHEP-Domain has been developed.

-Consensus pattern: [QY]-G-[DNEA]-x-[LIV]-[KR]-x(2)-K-x(2)-[KRNG]-[AS]-x(4)-[LIV]-[DENK]-x(2)-[IV]-x(2)-L-x(3)-K

[1] Cerini C., Kerjan P., Astier M., Gratecos D., Mirande M., Semeriva M.
EMBO J. 10:4267-4277(1991).
[2] Nada S., Chang P.K., Dignam J.D.
J. Biol. Chem. 268:7660-7667(1993).

719. (Worm family 8) Putative membrane protein
Analysis of protein domain families in Caenorhabditis elegans.
Sonnhammer EL, Durbin R;

Genomics 1997;46:200-216.

This family called family 8 in [1], may be a transmembrane protein

The specific function of this protein is unknown.

720. Xylose isomerase

Xylose isomerase (EC 5.3.1.5) [1] is an enzyme found in microorganisms which catalyzes the interconversion of D-xylose to D-xylulose. It can also isomerize D-ribose to D-ribulose and D-glucose to D-fructose. Xylose isomerase seems to require magnesium for its activity, while cobalt is necessary to stabilize the tetrameric structure of the enzyme. A number of residues are conserved in all known xylose isomerases.

Xylose isomerase also exists in plants [2] where it is homodimeric and is manganese-dependent.

Two signatures patterns for xylose isomerase have been developed. The first one is derived from a stretch of five conserved amino acids that includes a glutamic acid residue known to be one of the four residues involved in the binding of the magnesium ion [3]; this pattern also includes a lysine residue which is involved in the catalytic activity. The second pattern is derived from a conserved region in the N-terminal section of the enzyme that include an histidine residue which has been shown [4] to be involved in the catalytic mechanism of the enzyme.

-Consensus pattern: [LI]-E-P-K-P-x(2)-P
[E is a magnesium ligand]
[K is an active site residue]
-Consensus pattern: [FL]-H-D-x-D-[LIV]-x-[PD]-x-[GDE]
[H is an active site residue]

[1] Dauter Z., Dauter M., Hemker J., Witzel H., Wilson K.S.
FEBS Lett. 247:1-8(1989).

[2] Kristo P.A., Saarelainen R., Fagerstrom R., Aho S., Korhola M.
Eur. J. Biochem. 237:240-246(1996).
[3] Henrick K., Collyer C.A., Blow D.M.
J. Mol. Biol. 208:129-157(1989).
[4] Vangrysperre W., Ampe C., Kersters-Hilderson H., Tempst P.
Biochem. J. 263:195-199(1989).

721. XPG protein signatures. Xeroderma pigmentosum (XP) [1] is a human autosomal recessive disease, characterized by a high incidence of sunlight-induced skin cancer. People's skin cells with this condition are hypersensitive to ultraviolet light, due to defects in the incision step of DNA excision repair. There are a minimum of seven genetic complementation groups involved in this pathway: XP-A to XP-G. The defect in XP-G can be corrected by a 133 Kd nuclear protein called XPG (or XPGC) [2].XPG belongs to a family of proteins [2,3,4,5,6] that are composed of twomain subsets: - Subset 1, to which belongs XPG, RAD2 from budding yeast and rad13 from fission yeast. RAD2 and XPG are single-stranded DNA endonucleases [7,8]. XPG makes the 3'incision in human DNA nucleotide excision repair [9]. - Subset 2, to which belongs mouse and human FEN-1, rad2 from fission yeast, and RAD27 from budding yeast. FEN-1 is a structure-specific endonuclease. In addition to the proteins listed in the above groups, this family also includes: - Fission yeast exo1, a 5'->3' double-stranded DNA exonuclease that could act in a pathway that corrects mismatched base pairs. - Yeast EXO1 (DHS1), a protein with probably the same function as exo1. - Yeast DIN7.Sequence alignment of this family of proteins reveals that similarities are largely confined to two regions. The first is located at the N-terminal extremity (N-region) and corresponds to the first 95 to 105 amino acids. The second region is internal (I-region) and found towards the C-terminus; it spans about 140 residues and contains a highly conserved core of 27 amino acids that includes a conserved pentapeptide (E-A-[DE]-A-[QS]). It is possible that the conserved acidic residues are involved in the catalytic mechanism of DNA excision repair in XPG. The amino acids linking the N- and I-regions are not conserved; indeed, they are largely absent from proteins belonging to the second subset. Two signature patterns have been developed for these proteins. The first corresponds to the central part of the N-region, the second to part of the I-region and includes the putative catalytic core pentapeptide

Consensus pattern: [VI]-[KRE]-P-x-[FYIL]-V-F-D-G-x(2)-[PIL]-x-[LVC]-K-

Consensus pattern: [GS]-[LIVM]-[PER]-[FYS]-[LIVM]-x-A-P-x-E-A-[DE]-[PAS]- [QS]-[CLM]-

[1] Tanaka K., Wood R.D. Trends Biochem. Sci. 19:83-86(1994).[2] Scherly D., Nouspikel T., Corlet J., Ucla C., Bairoch A., Clarkson S.G. Nature 363:182-185(1993).[3] Carr A.M., Sheldrick K.S., Murray J.M., Al-Harithy R., Watts F.Z., Lehmann A.R. Nucleic Acids Res. 21:1345-1349(1993).[4] Murray J.M., Tavassoli M., Al-Harithy R., Sheldrick K.S., Lehmann A.R., Carr A.M., Watts F.Z. Mol. Cell. Biol. 14:4878-4888(1994).[5] Harrington J.J., Lieber M.R. Genes Dev. 8:1344-1355(1994).[6] Szankasi P., Smith G.R. Science 267:1166-1169(1995).[7] Habraken Y., Sung P., Prakash L., Prakash S. Nature 366:365-368(1993).[8] O'Donovan A., Scherly D., Clarkson S.G., Wood R.D. J. Biol. Chem. 269:15965-15968(1994).[9] O'Donovan A., Davies A.A., Moggs J.G., West S.C., Wood R.D. Nature 371:432-435(1994).

722. Xanthine/uracil permeases family

The following transport proteins which are involved in the uptake of xanthine or uracil are evolutionary related [1]:

- Uric uric acid-xanthine permease (gene uapA) from Aspergillus nidulans.
- Purine permease (gene uapC) from Aspergillus nidulans.
- Xanthine permease from Bacillus subtilis (gene pbuX).
- Uracil permease from Escherichia coli (gene uraA) [2] and Bacillus (gene pyrP).
- Hypothetical protein ycdG from Escherichia coli.
- Hypothetical protein ygfO from Escherichia coli.
- Hypothetical protein ygfU from Escherichia coli.
- Hypothetical protein yicE from Escherichia coli.
- Hypothetical protein yunJ from Bacillus subtilis.
- Hypothetical protein yunK from Bacillus subtilis.

09555256.061900

They are proteins of from 430 to 595 residues that seem to contain 12 transmembrane domains.

The best conserved region which corresponds with what seems to be the tenth transmembrane domain has been selected as a signature pattern.

-Consensus pattern: [LIVM]-P-x-[PASIF]-V-[LIVM]-G-G-x(4)-[LIVM]-[FY]-[GSA]-x-[LIVM]-x(3)-G

[1] Diallinas G., Gorfinkiel L., Arst G., Cecchetto G., Scazzocchio C.
J. Biol. Chem. 270:8610-8622(1995).
[2] Andersen P.S., Frees D., Fast R., Mygind B.
J. Bacteriol. 177:2008-2013(1995).

09556306 .061600

723. Hypothetical yabO/yceC/sfhB family

The following proteins, which seems to belong to a family of pseudouridine synthases (EC 4.2.1.70) [1] have been shown to share regions of similarities:

- Escherichia coli and Haemophilus influenzae ribosomal large subunit pseudouridine synthase A (gene rluA). It is responsible for synthesis of pseudouridine from uracil-746 IN 23S rRNA.
- Escherichia coli and Haemophilus influenzae ribosomal large subunit pseudouridine synthase C (gene rluC). It is responsible for synthesis of pseudouridine from uracil at positions 955, 2504 and 2580 in 23S rRNA.
- Escherichia coli protein and homologs in other bacteria large subunit pseudouridine synthase D (gene rluD).
- Yeast DRAP deaminase (gene RIB2).
- Escherichia coli hypothetical protein yqcB and HI1435, the corresponding Haemophilus influenzae protein.
- Haemophilus influenzae hypothetical protein HI0042.
- Aquifex aeolicus hypothetical protein AQ_1758.
- Bacillus subtilis hypothetical protein yhcT.
- Bacillus subtilis hypothetical protein yjbO.
- Bacillus subtilis hypothetical protein ylyB.

- Helicobacter pylori hypothetical protein HP0347.
- Helicobacter pylori hypothetical protein HP0745.
- Helicobacter pylori hypothetical protein HP0956.
- Mycoplasma genitalium hypothetical protein MG209.
- Mycoplasma genitalium hypothetical protein MG370.
- Synechocystis strain PCC 6803 hypothetical protein slr1592.
- Synechocystis strain PCC 6803 hypothetical protein slr1629.
- Yeast hypothetical protein YDL036c.
- Yeast hypothetical protein YGR169c.
- Fission yeast hypothetical protein SpAC18B11.02c.
- Caenorhabditis elegans hypothetical protein K07E8.7.

These are proteins of from 21 to 50 Kd which contain a number of conserved regions in their central section. They can be picked up in the database by the following highly conserved pattern.

-Consensus pattern: [LIVCA]-[NHYT]-R-[LI]-D-x(2)-T-[STA]-G-[LIVAGC]-
[LIVMF](2)-[LIVMFGC]-[SGTACV]

[1] Conrad J., Sun D., Englund N., Ofengand J.
J. Biol. Chem. 273:18562-18566(1998).

In addition, the following bacterial proteins, which seems to belong to a family of pseudouridine synthases (EC 4.2.1.70) [1] also have been shown to share regions of similarities:

- Escherichia coli and Haemophilus influenzae 16S pseudouridylate 516 synthase (EC 4.2.1.70) (gene: rsuA). This enzyme is responsible for the formation of pseudouridine from uracil-516 in 16S ribosomal RNA.
- Escherichia coli hypothetical protein yciL and HI1199, the corresponding Haemophilus influenzae protein.
- Escherichia coli hypothetical protein yjbC.
- Escherichia coli hypothetical protein ymfC and HI0694, the corresponding

Haemophilus influenzae protein.

- Aquifex aeolicus hypothetical protein AQ_554.
- Aquifex aeolicus hypothetical protein AQ_1464.
- Bacillus subtilis hypothetical protein ypuL.
- Bacillus subtilis hypothetical protein ytzF.
- Borrelia burgdorferi hypothetical protein BB0129.
- Helicobacter pylori hypothetical protein HP1459.
- Synechocystis strain PCC 6803 hypothetical protein slr0361.
- Synechocystis strain PCC 6803 hypothetical protein slr0612.

These are proteins of from 25 to 40 Kd which contain a number of conserved regions in their central section. They can be picked up in the database by the following highly conserved pattern.

-Consensus pattern: G-R-L-D-x(2)-[STA]-x-G-[LIVFA]-[LIVMF](3)-[ST]-[DNST]

[1] Wrzesinski J., Bakin A., Nurse K., Lane B.G., Ofengand J.
Biochemistry 34:8904-8913(1995).

09553296.051500

724. Zinc finger present in dystrophin, CBP/p300
ZZ in dystrophin binds calmodulin
Putative zinc finger; binding not yet shown.

725. Zinc carboxypeptidase
There are a number of different types of zinc-dependent carboxypeptidases (EC 3.4.17.-) [1,2]. All these enzymes seem to be structurally and functionally related. The enzymes that belong to this family are listed below.

- Carboxypeptidase A1 (EC 3.4.17.1), a pancreatic digestive enzyme that can removes all C-terminal amino acids with the exception of Arg, Lys and Pro.
- Carboxypeptidase A2 (EC 3.4.17.15), a pancreatic digestive enzyme with a

specificity similar to that of carboxypeptidase A1, but with a preference for bulkier C-terminal residues.

- Carboxypeptidase B (EC 3.4.17.2), also a pancreatic digestive enzyme, but that preferentially removes C-terminal Arg and Lys.
- Carboxypeptidase N (EC 3.4.17.3) (also known as arginine carboxypeptidase), a plasma enzyme which protects the body from potent vasoactive and inflammatory peptides containing C-terminal Arg or Lys (such as kinins or anaphylatoxins) which are released into the circulation.
- Carboxypeptidase H (EC 3.4.17.10) (also known as enkephalin convertase or carboxypeptidase E), an enzyme located in secretory granules of pancreatic islets, adrenal gland, pituitary and brain. This enzyme removes residual C-terminal Arg or Lys remaining after initial endoprotease cleavage during prohormone processing.
- Carboxypeptidase M (EC 3.4.17.12), a membrane bound Arg and Lys specific enzyme.
  It is ideally situated to act on peptide hormones at local tissue sites where it could control their activity before or after interaction with specific plasma membrane receptors.
- Mast cell carboxypeptidase (EC 3.4.17.1), an enzyme with a specificity to carboxypeptidase A, but found in the secretory granules of mast cells.
- Streptomyces griseus carboxypeptidase (Cpase SG) (EC 3.4.17.-) [3], which combines the specificities of mammalian carboxypeptidases A and B.
- Thermoactinomyces vulgaris carboxypeptidase T (EC 3.4.17.18) (CPT) [4], which also combines the specificities of carboxypeptidases A and B.
- AEBP1 [5], a transcriptional repressor active in preadipocytes. AEBP1 seems to regulate transcription by cleavage of other transcriptional proteins.
- Yeast hypothetical protein YHR132c.

All of these enzymes bind an atom of zinc. Three conserved residues are implicated in the binding of the zinc atom: two histidines and a glutamic acid Two signature patterns which contain these three zinc-ligands have been derived.

-Consensus pattern: [PK]-x-[LIVMFY]-x-[LIVMFY]-x(4)-H-[STAG]-x-E-x-[LIVM]-

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[STAG]-x(6)-[LIVMFYTA]

[H and E are zinc ligands]

-Consensus pattern: H-[STAG]-x(3)-[LIVME]-x(2)-[LIVMFYW]-P-[FYW]

[H is a zinc ligand]

[1] Tan F., Chan S.J., Steiner D.F., Schilling J.W., Skidgel R.A.
J. Biol. Chem. 264:13165-13170(1989).

[2] Reynolds D.S., Stevens R.L., Gurley D.S., Lane W.S., Austen K.F., Serafin W.E.
J. Biol. Chem. 264:20094-20099(1989).

[3] Narahashi Y.
J. Biochem. 107:879-886(1990).

[4] Teplyakov A., Polyakov K., Obmolova G., Strokopytov B., Kuranova I., Osterman A.L., Grishin N.V., Smulevitch S.V., Zagnitko O.P., Galperina O.V., Matz M.V., Stepanov V.M.
Eur. J. Biochem. 208:281-288(1992).

[5] He G.-P., Muise A., Li A.W., Ro H.-S.
Nature 378:92-96(1995).

[6] Hourdou M.-L., Guinand M., Vacheron M.J., Michel G., Denoroy L., Duez C.M., Englebert S., Joris B., Weber G., Ghuysen J.-M.
Biochem. J. 292:563-570(1993).

[7] Rawlings N.D., Barrett A.J.
Meth. Enzymol. 248:183-228(1995).

726. Zinc finger, C2H2 type

The C2H2 zinc finger is the classical zinc finger domain. The two conserved cysteines and histidines co-ordinate a zinc ion. The following pattern describes the zinc finger.

#-X-C-X(1-5)-C-X3-#-X5-#-X2-H-X(3-6)-[H/C]

Where X can be any amino acid, and numbers in brackets indicate the number of residues. The positions marked # are those that are important for the stable fold of the zinc

finger. The final position can be either his or cys.
The C2H2 zinc finger is composed of two short beta strands followed by an alpha helix. The amino terminal part of the helix binds the major groove in DNA binding zinc fingers.

'Zinc finger' domains [1-5] are nucleic acid-binding protein structures first identified in the Xenopus transcription factor TFIIIA. These domains have since been found in numerous nucleic acid-binding proteins. A zinc finger domain is composed of 25 to 30 amino-acid residues. There are two cysteine or histidine residues at both extremities of the domain, which are involved in the tetrahedral coordination of a zinc atom. It has been proposed that such a domain interacts with about five nucleotides. A schematic representation of a zinc finger domain is shown below:

```
  x x
  x   x
 x x
 x x
 x x
 x x
  C   H
 x  \ /  x
  x  Zn  x
 x /   \ x
  C   H
  x x x x x  x x x x x
```

Many classes of zinc fingers are characterized according to the number and positions of the histidine and cysteine residues involved in the zinc atom coordination. In the first class to be characterized, called C2H2, the first pair of zinc coordinating residues are cysteines, while the second pair are histidines. A number of experimental reports have demonstrated the zinc-dependent DNA or RNA binding property of some members of this class.

Some of the proteins known to include C2H2-type zinc fingers are listed below. The number of zinc finger regions found in each of these proteins are indicated between brackets; a '+' symbol indicates that only partial sequence data is available and that additional finger domains may be present.

- Saccharomyces cerevisiae: ACE2 (3), ADR1 (2), AZF1 (4), FZF1 (5), MIG1 (2), MSN2 (2), MSN4 (2), RGM1 (2), RIM1 (3), RME1 (3), SFP1 (2), SSL1 (1), STP1 (3), SWI5 (3), VAC1 (1) and ZMS1 (2).
- Emericella nidulans: brlA (2), creA (2).
- Drosophila: AEF-1 (4), Cf2 (7), ci-D (5), Disconnected (2), Escargot (5), Glass (5), Hunchback (6), Kruppel (5), Kruppel-H (4+), Odd-skipped (4), Odd-paired (4), Pep (3), Snail (5), Spalt-major (7), Serependity locus beta (6), delta (7), h-1 (8), Suppressor of hairy wing su(Hw) (12), Suppressor of variegation suvar(3)7 (5), Teashirt (3) and Tramtrack (2).
- Xenopus: transcription factor TFIIIA (9), p43 from RNP particle (9), Xfin (37 !!), Xsna (5), gastrula XlcGF5.1 to XlcGF71.1 (from 4+ to 11+), Oocyte XlcOF2 to XlcOF22 (from 7 to 12).
- Mammalian: basonuclin (6), BCL-6/LAZ-3 (6), erythroid krueppel-like transcription factor (3), transcription factors Sp1 (3), Sp2 (3), Sp3 (3) and Sp(4) 3, transcriptional repressor YY1 (4), Wilms' tumor protein (4), EGR1/Krox24 (3), EGR2/Krox20 (3), EGR3/Pilot (3), EGR4/AT133 (4), Evi-1 (10), GLI1 (5), GLI2 (4+), GLI3 (3+), HIV-EP1/ZNF40 (4), HIV-EP2 (2), KR1 (9+), KR2 (9), KR3 (15+), KR4 (14+), KR5 (11+), HF.12 (6+), REX-1 (4), ZfX (13), ZfY (13), Zfp-35 (18), ZNF7 (15), ZNF8 (7), ZNF35 (10), ZNF42/MZF-1 (13), ZNF43 (22), ZNF46/Kup (2), ZNF76 (7), ZNF91 (36), ZNF133 (3).

In addition to the conserved zinc ligand residues it has been shown [6] that a number of other positions are also important for the structural integrity of the C2H2 zinc fingers. The best conserved position is found four residues after the second cysteine; it is generally an aromatic or aliphatic residue.

-Consensus pattern: C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H

[The two C's and two H's are zinc ligands]

[1] Klug A., Rhodes D.
Trends Biochem. Sci. 12:464-469(1987).
[2] Evans R.M., Hollenberg S.M.
Cell 52:1-3(1988).
[3] Payre F., Vincent A.
FEBS Lett. 234:245-250(1988).
[4] Miller J., McLachlan A.D., Klug A.
EMBO J. 4:1609-1614(1985).
[5] Berg J.M.
Proc. Natl. Acad. Sci. U.S.A. 85:99-102(1988).
[6] Rosenfeld R., Margalit H.
J. Biomol. Struct. Dyn. 11:557-570(1993).

09555326.091500

727. Zinc finger, C3HC4 type (RING finger)

A number of eukaryotic and viral proteins contain a conserved cysteine-rich domain of 40 to 60 residues (called C3HC4 zinc-finger or 'RING' finger) [1] that binds two atoms of zinc, and is probably involved in mediating protein-protein interactions. The 3D structure of the zinc ligation system is unique to the RING domain and is refered to as the "cross-brace" motif. The spacing of the cysteines in such a domain is C-x(2)-C-x(9 to 39)-C-x(1 to 3)-H-x(2 to 3)-C-x(2)-C-x(4 to 48)-C-x(2)-C.

Proteins currently known to include the C3HC4 domain are listed below (references are only provided for recently determined sequences).

- Mammalian V(D)J recombination activating protein (gene RAG1). RAG1 activates the rearrangement of immunoglobulin and T-cell receptor genes.
- Mouse rpt-1. Rpt-1 is a trans-acting factor that regulates gene expression directed by the promoter region of the interleukin-2 receptor alpha chain or the LTR promoter region of HIV-1.

- Human rfp. Rfp is a developmentally regulated protein that may function in male germ cell development. Recombination of the N-terminal section of rfp with a protein tyrosine kinase produces the ret transforming protein.
- Human 52 Kd Ro/SS-A protein. A protein of unknown function from the Ro/SS-A ribonucleoprotein complex. Sera from patients with systemic lupus erythematosus or primary Sjogren's syndrome often contain antibodies that react with the Ro proteins.
- Human histocompatibility locus protein RING1.
- Human PML, a probable transcription factor. Chromosomal translocation of PML with retinoic receptor alpha creates a fusion protein which is the cause of acute promyelocytic leukemia (APL).
- Mammalian breast cancer type 1 susceptibility protein (BRCA1) [E1].
- Mammalian cbl proto-oncogene.
- Mammalian bmi-1 proto-oncogene.
- Vertebrate CDK-activating kinase (CAK) assembly factor MAT1, a protein that stabilizes the complex between the CDK7 kinase and cyclin H (MAT1 stands for 'Menage A Trois').
- Mammalian mel-18 protein. Mel-18 which is expressed in a variety of tumor cells is a transcriptional repressor that recognizes and bind a specific DNA sequence.
- Mammalian peroxisome assembly factor-1 (PAF-1) (PMP35), which is somewhat involved in the biogenesis of peroxisomes. In humans, defects in PAF-1 are responsible for a form of Zellweger syndrome, an autosomal recessive disorder associated with peroxisomal deficiencies.
- Human MAT1 protein, which interacts with the CDK7-cyclin H complex.
- Human RING1 protein.
- Xenopus XNF7 protein, a probable transcription factor.
- Trypanosoma protein ESAG-8 (T-LR), which may be involved in the postranscriptional regulation of genes in VSG expression sites or may interact with adenylate cyclase to regulate its activity.
- Drosophila proteins Posterior Sex Combs (Psc) and Suppressor two of zeste (Su(z)2). The two proteins belong to the Polycomb group of genes needed to maintain the segment-specific repression of homeotic selector genes.

- Drosophila protein male-specific msl-2, a DNA-binding protein which is involved in X chromosome dosage compensation (the elevation of transcription of the male single X chromosome).
- Arabidopsis thaliana protein COP1 which is involved in the regulation of photomorphogenesis.
- Fungal DNA repair proteins RAD5, RAD16, RAD18 and rad8.
- Herpesviruses trans-acting transcriptional protein ICP0/IE110. This protein which has been characterized in many different herpesviruses is a trans-activator and/or -repressor of the expression of many viral and cellular promoters.
- Baculoviruses protein CG30.
- Baculoviruses major immediate early protein (PE-38).
- Baculoviruses immediate-early regulatory protein IE-N/IE-2.
- Caenorhabditis elegans hypothetical proteins F54G8.4, R05D3.4 and T02C1.1.
- Yeast hypothetical proteins YER116c and YKR017c.

09555325.061500

The central region of the domain was selected as a signature pattern for the C3HC4 finger.

-Consensus pattern: C-x-H-x-[LIVMFY]-C-x(2)-C-[LIVMYA]

[1] Borden K.L.B., Freemont P.S.
Curr. Opin. Struct. Biol. 6:395-401(1996).

728. Zinc finger C-x8-C-x5-C-x3-H type (and similar).

729. Zinc finger, CCHC class

A family of CCHC zinc fingers, mostly from retroviral gag proteins (nucleocapsid). Prototype structure is from HIV. Also contains members involved in eukaryotic gene regulation, such as C. elegans GLH-1.

Structure is an 18-residue zinc finger; no examples of indels in the alignment.

730. Zn-finger in Ran binding protein and others.

731. AN1-like Zinc finger

Zinc finger at the C-terminus of An1 Swiss:Q91889, a ubiquitin-like protein in Xenopus laevis. The following pattern describes the zinc finger. C-X2-C-X(9-12)-C-X(1-2)-C-X4-C-X2-H-X5-H-X-C Where X can be any amino acid, and numbers in brackets indicate the number of residues.

[1] Linnen JM, Bailey CP, Weeks DL; Gene 1993;128:181-188.

09955226.060100

732. 14-3-3 proteins

Structure of a 14-3-3 protein and implications for coordination of multiple signalling pathways.
Xiao B, Smerdon SJ, Jones DH, Dodson GG, Soneji Y, Aitken A, Gamblin SJ;
Nature 1995;376:188-191.
Crystal structure of the zeta isoform of the 14-3-3 protein.
Liu D, Bienkowska J, Petosa C, Collier RJ, Fu H, Liddington R;
Nature 1995;376:191-194.

Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine.
Muslin AJ, Tanner JW, Allen PM, Shaw AS;
Cell 1996;84:889-897.

The 14-3-3 protein binds its target proteins with a common site located towards the C-terminus.

Ichimura T, Ito M, Itagaki C, Takahashi M, Horigome T, Omata S, Ohno S, Isobe T
FEBS Lett 1997;413:273-276.

Molecular evolution of the 14-3-3 protein family.
Wang W, Shakes DC
J Mol Evol 1996;43:384-398.
Function of 14-3-3 proteins.
Jin DY, Lyu MS, Kozak CA, Jeang KT
Nature 1996;382:308-308.

09525756.062500

The 14-3-3 proteins [1,2,3] are a family of closely related acidic homodimeric proteins of about 30 Kd which were first identified as being very abundant in mammalian brain tissues and located preferentially in neurons. The 14-3-3 proteins seem to have multiple biological activities and play a key role in signal transduction pathways and the cell cycle. They interacts with kinases such as PKC or Raf-1; they seem to also function as protein-kinase dependent activators of tyrosine and tryptophan hydroxylases and in plants they are associated with a complex that binds to the G-box promoter elements.

The 14-3-3 family of proteins are ubiquitously found in all eukaryotic species studied and have been sequenced in fungi (yeast BMH1 and BMH2, fission yeast rad24 and rad25), plants, Drosophila, and vertebrates. The sequences of the 14-3-3 proteins are extremely well conserved. Two highly conserved regions have been selected as signature patterns: the first is a peptide of 11 residues located in the N-terminal section; the second, a 20 amino acid region located in the C-terminal section.

-Consensus pattern: R-N-L-[LIV]-S-[VG]-[GA]-Y-[KN]-N-[IVA]
-Consensus pattern: Y-K-[DE]-S-T-L-I-[IM]-Q-L-[LF]-[RHC]-D-N-[LF]-T-[LS]-W-[TAN]-[SAD]

[1] Aitken A.

Trends Biochem. Sci. 20:95-97(1995).

[2] Morrison D.
Science 266:56-57(1994).

[3] Xiao B., Smerdon S.J., Jones D.H., Dodson G.G., Soneji Y., Aitken A., Gamblin S.J.
Nature 376:188-191(1995).

733. D-isomer specific 2-hydroxyacid dehydrogenases (2 Hacid DH)

This Pfam covers the Formate dehydrogenase, D-glycerate dehydrogenase and D-lactate dehydrogenase families in SCOP. A number of NAD-dependent 2-hydroxyacid dehydrogenases which seem to be specific for the D-isomer of their substrate have been shown [1,2,3,4] to be functionally and structurally related. These enzymes are listed below.

- D-lactate dehydrogenase (EC 1.1.1.28), a bacterial enzyme which catalyzes the reduction of D-lactate to pyruvate.
- D-glycerate dehydrogenase (EC 1.1.1.29) (NADH-dependent hydroxypyruvate reductase), a plant leaf peroxisomal enzyme that catalyzes the reduction of hydroxypyruvate to glycerate. This reaction is part of the glycolate pathway of photorespiration.
- D-glycerate dehydrogenase from the bacteria Hyphomicrobium methylovorum and Methylobacterium extorquens.
- 3-phosphoglycerate dehydrogenase (EC 1.1.1.95), a bacterial enzyme that catalyzes the oxidation of D-3-phosphoglycerate to 3-phosphohydroxypyruvate. This reaction is the first committed step in the 'phosphorylated' pathway of serine biosynthesis.
- Erythronate-4-phosphate dehydrogenase (EC 1.1.1.-) (gene pdxB), a bacterial enzyme involved in the biosynthesis of pyridoxine (vitamin B6).
- D-2-hydroxyisocaproate dehydrogenase (EC 1.1.1.-) (D-hicDH), a bacterial enzyme that catalyzes the reversible and stereospecific interconversion between 2-ketocarboxylic acids and D-2-hydroxy-carboxylic acids.
- Formate dehydrogenase (EC 1.2.1.2) (FDH) from the bacteria Pseudomonas sp. 101 and various fungi [5].
- Vancomycin resistance protein vanH from Enterococcus faecium; this protein is a D-specific alpha-keto acid dehydrogenase involved in the formation of a

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peptidoglycan which does not terminate by D-alanine thus preventing vancomycin binding.

- Escherichia coli hypothetical protein ycdW.
- Escherichia coli hypothetical protein yiaE.
- Haemophilus influenzae hypothetical protein HI1556.
- Yeast hypothetical protein YER081w.
- Yeast hypothetical protein YIL074w.

All these enzymes have similar enzymatic activities and are structurally related. Three of the most conserved regions of these proteins have been selected to develop patterns. The first pattern is based on a glycine-rich region located in the central section of these enzymes; this region probably corresponds to the NAD-binding domain. The two other patterns contain a number of conserved charged residues, some of which may play a role in the catalytic mechanism.

-Consensus pattern: [LIVMA]-[AG]-[IVT]-[LIVMFY]-[AG]-x-G-[NHKRQGSAC]-[LIV]-G-x(13,14)-[LIVfMT]-x(2)-[FYwCTH]-[DNSTK]
-Consensus pattern: [LIVMFYWA]-[LIVFYWC]-x(2)-[SAC]-[DNQHR]-[IVFA]-[LIVF]-x-[LIVF]-[HNI]-x-P-x(4)-[STN]-x(2)-[LIVMF]-x-[GSDN]
-Consensus pattern: [LMFATC]-[KPQ]-x-[GSTDN]-x-[LIVMFYWR]-[LIVMFYW](2)-N-x-[STAGC]-R-[GP]-x-[LIVH]-[LIVMC]-[DNV]

[1] Grant G.A. Biochem. Biophys. Res. Commun. 165:1371-1374(1989).
[2] Kochhar S., Hunziker P., Leong-Morgenthaler P.M., Hottinger H. Biochem. Biophys. Res. Commun. 184:60-66(1992).
[3] Ohta T., Taguchi H. J. Biol. Chem. 266:12588-12594(1991).
[4] Goldberg J.D., Yoshida T., Brick P. J. Mol. Biol. 236:1123-1140(1994).
[5] Popov V.O., Lamzin V.S. Biochem. J. 301:625-643(1994).

734. 2-oxo acid dehydrogenases acyltransferase (catalytic domain)
Refined crystal structure of the catalytic domain of dihysrolipoyl transacetylase (E2P) from azotobacter vineelandii at 2.6 angstroms resolution.

Mattevi A, Obmolova G, Kalk KH, Westphal AH, De Kok A, Hol WG; J Mol Biol 1993;230:1183-1199.
These proteins contain one to three copies of a lipoyl binding domain followed by the catalytic domain.

735. 3-beta hydroxysteriod dehydrogenase/isomerase family
Structure and tissue-specific expression of 3 beta-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase genes in human and rat classical and peripheral steroidogenic tissues.
Labrie F, Simard J, Luu-The V, Pelletier G, Belanger A, Lachance Y, Zhao HF, Labrie C, Breton N, de Launoit Y, et al
J Steroid Biochem Mol Biol 1992;41:421-435.
The enzyme 3 beta-hydroxysteroid dehydrogenase/5-ene-4-ene isomerase (3 beta-HSD) catalyzes the oxidation and isomerization of 5-ene-3 beta-hydroxypregnene and 5-ene-hydroxyandrostene steroid precursors into the corresponding 4-ene-ketosteroids necessary for the formation of all classes of steroid hormones.

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736. 3-hydroxyacyl-CoA dehydrogenase
This family also includes lambda crystallin.
Structure of L-3-hydroxyacyl-coenzyme A dehydrogenase: preliminary chain tracing at 2.8-A resolution.
Birktoft JJ, Holden HM, Hamlin R, Xuong NH, Banaszak LJ;
Proc Natl Acad Sci U S A 1987;84:8262-8266.

3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (HCDH) [1] is an enzyme involved in fatty acid metabolism, it catalyzes the reduction of 3-hydroxyacyl-CoA to 3-oxoacyl-CoA. Most eukaryotic cells have 2 fatty-acid beta-oxidation systems, one located in mitochondria and the other in peroxisomes. In peroxisomes 3-hydroxyacyl-CoA dehydrogenase forms, with enoyl-CoA hydratase (ECH) and

3,2-trans-enoyl-CoA isomerase (ECI) a multifunctional enzyme where the N-terminal domain bears the hydratase/isomerase activities and the C-terminal domain the dehydrogenase activity. There are two mitochondrial enzymes: one which is monofunctional and the other which is, like its peroxisomal counterpart, multifunctional.

In Escherichia coli (gene fadB) and Pseudomonas fragi (gene faoA) HCDH is part of a multifunctional enzyme which also contains an ECH/ECI domain as well as a 3-hydroxybutyryl-CoA epimerase domain [2].

The other proteins structurally related to HCDH are:

- Bacterial 3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157) which reduces 3-hydroxybutanoyl-CoA to acetoacetyl-CoA [3].
- Eye lens protein lambda-crystallin [4], which is specific to lagomorphes (such as rabbit).

There are two major region of similarities in the sequences of proteins of the HCDH family, the first one located in the N-terminal, corresponds to the NAD-binding site, the second one is located in the center of the sequence. A signature pattern has been derived from this central region.

-Consensus pattern: [DNE]-x(2)-[GA]-F-[LIVMFY]-x-[NT]-R-x(3)-[PA]-[LIVMFY](2)-x(5)-[LIVMFYCT]-[LIVMFY]-x(2)-[GV]

[1] Birktoff J.J., Holden H.M., Hamlin R., Xuong N.-H., Banaszak L.J.
Proc. Natl. Acad. Sci. U.S.A. 84:8262-8266(1987).
[2] Nakahigashi K., Inokuchi H.
Nucleic Acids Res. 18:4937-4937(1990).
[3] Mullany P., Clayton C.L., Pallen M.J., Slone R., Al-Saleh A., Tabaqchali S.
FEMS Microbiol. Lett. 124:61-67(1994).
[4] Mulders J.W.M., Hendriks W., Blankesteijn W.M., Bloemendal H.,

de Jong W.W.
J. Biol. Chem. 263:15462-15466(1988).

737. 60s Acidic ribosomal protein
Proteins P1, P2, and P0, components of the eukaryotic ribosome stalk. New structural and functional aspects.
Remacha M, Jimenez-Diaz A, Santos C, Briones E, Zambrano R, Rodriguez Gabriel MA, Guarinos E, Ballesta JP;
Biochem Cell Biol 1995;73:959-968.
This family includes archaebacterial L12, eukaryotic P0, P1 and P2.

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738. 6-phosphogluconate dehydrogenases
6-phosphogluconate dehydrogenase (EC 1.1.1.44) (6PGD) catalyzes the third step in the hexose monophosphate shunt, the decarboxylating reduction of 6-phosphogluconate in to ribulose 5-phosphate.

Prokaryotic and eukaryotic 6PGD are proteins of about 470 amino acids whose sequence are highly conserved [1]. A region which has been shown [2], from studies of the sheep 6PGD tertiary structure, to be involved in the binding of 6-phosphogluconate has been selected as a signature pattern.

-Consensus pattern: [LIVM]-x-D-x(2)-[GA]-[NQS]-K-G-T-G-x-W

[1] Reizer A., Deutscher J., Saier M.H. Jr., Reizer J.
Mol. Microbiol. 5:1081-1089(1991).
[2] Adams M.J., Archibald I.G., Bugg C.E., Carne A., Gover S., Helliwell J.R., Pickersgill R.W., White S.W.
EMBO J. 2:1009-1014(1983).

739. (7tm 1) G-protein coupled receptors [1 to 4,E1,E2] (also called R7G) are an extensive

group of hormones, neurotransmitters, odorants and light receptors which transduce extracellular signals by interaction with guanine nucleotide-binding (G) proteins. The receptors that are currently known to belong to this family are listed below.

- 5-hydroxytryptamine (serotonin) 1A to 1F, 2A to 2C, 4, 5A, 5B, 6 and 7 [5].
- Acetylcholine, muscarinic-type, M1 to M5.
- Adenosine A1, A2A, A2B and A3 [6].
- Adrenergic alpha-1A to -1C; alpha-2A to -2D; beta-1 to -3 [7].
- Angiotensin II types I and II.
- Bombesin subtypes 3 and 4.
- Bradykinin B1 and B2.
- c3a and C5a anaphylatoxin.
- Cannabinoid CB1 and CB2.
- Chemokines C-C CC-CKR-1 to CC-CKR-8.
- Chemokines C-X-C CXC-CKR-1 to CXC-CKR-4.
- Cholecystokinin-A and cholecystokinin-B/gastrin.
- Dopamine D1 to D5 [8].
- Endothelin ET-a and ET-b [9].
- fMet-Leu-Phe (fMLP) (N-formyl peptide).
- Follicle stimulating hormone (FSH-R) [10].
- Galanin.
- Gastrin-releasing peptide (GRP-R).
- Gonadotropin-releasing hormone (GNRH-R).
- Histamine H1 and H2 (gastric receptor I).
- Lutropin-choriogonadotropic hormone (LSH-R) [10].
- Melanocortin MC1R to MC5R.
- Melatonin.
- Neuromedin B (NMB-R).
- Neuromedin K (NK-3R).
- Neuropeptide Y types 1 to 6.
- Neurotensin (NT-R).
- Octopamine (tyramine), from insects.

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- Odorants [11].
- Opioids delta-, kappa- and mu-types [12].
- Oxytocin (OT-R).
- Platelet activating factor (PAF-R).
- Prostacyclin.
- Prostaglandin D2.
- Prostaglandin E2, EP1 to EP4 subtypes.
- Prostaglandin F2.
- Purinoreceptors (ATP) [13].
- Somatostatin types 1 to 5.
- Substance-K (NK-2R).
- Substance-P (NK-1R).
- Thrombin.
- Thromboxane A2.
- Thyrotropin (TSH-R) [10].
- Thyrotropin releasing factor (TRH-R).
- Vasopressin V1a, V1b and V2.
- Visual pigments (opsins and rhodopsin) [14].
- Proto-oncogene mas.
- A number of orphan receptors (whose ligand is not known) from mammals and birds.
- Caenorhabditis elegans putative receptors C06G4.5, C38C10.1, C43C3.2, T27D1.3 and ZC84.4.
- Three putative receptors encoded in the genome of cytomegalovirus: US27, US28, and UL33.
- ECRF3, a putative receptor encoded in the genome of herpesvirus saimiri.

The structure of all these receptors is thought to be identical. They have seven hydrophobic regions, each of which most probably spans the membrane. The N-terminus is located on the extracellular side of the membrane and is often glycosylated, while the C-terminus is cytoplasmic and generally phosphorylated. Three extracellular loops alternate with three intracellular loops to link the seven transmembrane regions. Most, but not all of these

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receptors, lack a signal peptide. The most conserved parts of these proteins are the transmembrane regions and the first two cytoplasmic loops. A conserved acidic-Arg-aromatic triplet is present in the N-terminal extremity of the second cytoplasmic loop [15] and could be implicated in the interaction with G proteins.

To detect this widespread family of proteins, a pattern that contains the conserved triplet and that also spans the major part of the third transmembrane helix has been developed.

-Consensus pattern: [GSTALIVMFYWC]-[GSTANCPDE]-{EDPKRH}-x(2)-[LIVMNQGA]-x(2)-
[LIVMFT]-[GSTANC]-[LIVMFYWSTAC]-[DENH]-R-[FYWCSH]-x(2)-[LIVM]

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[1] Strosberg A.D.
Eur. J. Biochem. 196:1-10(1991).
[2] Kerlavage A.R.
Curr. Opin. Struct. Biol. 1:394-401(1991).
[3] Probst W.C., Snyder L.A., Schuster D.I., Brosius J., Sealfon S.C.
DNA Cell Biol. 11:1-20(1992).
[4] Savarese T.M., Fraser C.M.
Biochem. J. 283:1-9(1992).
[5] Branchek T.
Curr. Biol. 3:315-317(1993).
[6] Stiles G.L.
J. Biol. Chem. 267:6451-6454(1992).
[7] Friell T., Kobilka B.K., Lefkowitz R.J., Caron M.G.
Trends Neurosci. 11:321-324(1988).
[8] Stevens C.F.
Curr. Biol. 1:20-22(1991).
[9] Sakurai T., Yanagisawa M., Masaki T.
Trends Pharmacol. Sci. 13:103-107(1992).

[10] Salesse R., Remy J.J., Levin J.M., Jallal B., Garnier J.
Biochimie 73:109-120(1991).
[11] Lancet D., Ben-Arie N.
Curr. Biol. 3:668-674(1993).
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Trends Neurosci. 17:89-93(1994).
[13] Barnard E.A., Burnstock G., Webb T.E.
Trends Pharmacol. Sci. 15:67-70(1994).
[14] Applebury M.L., Hargrave P.A.
Vision Res. 26:1881-1895(1986).
[15] Attwood T.K., Eliopoulos E.E., Findlay J.B.C.
Gene 98:153-159(1991).

(7tm 1) Visual pigments (opsins) retinal binding site

Visual pigments [1,2] are the light-absorbing molecules that mediate vision. They consist of an apoprotein, opsin, covalently linked to the chromophore cis-retinal. Vision is effected through the absorption of a photon by cis-retinal which is isomerized to trans-retinal. This isomerization leads to a change of conformation of the protein. Opsins are integral membrane proteins with seven transmembrane regions that belong to family 1 of G-protein coupled receptors.

In vertebrates four different pigments are generally found. Rod cells, which mediate vision in dim light, contain the pigment rhodopsin. Cone cells, which function in bright light, are responsible for color vision and contain three or more color pigments (for example, in mammals: red, blue and green).

In Drosophila, the eye is composed of 800 facets or ommatidia. Each ommatidium contains eight photoreceptor cells (R1-R8): the R1 to R6 cells are outer cells, R7 and R8 inner cells. Each of the three types of cells (R1-R6, R7 and R8) expresses a specific opsin.

Proteins evolutionary related to opsins include squid retinochrome, also known as retinal photoisomerase, which converts various isomers of retinal into 11-cis retinal and mammalian retinal pigment epithelium (RPE) RGR [3], a protein that may also act in retinal isomerization.

The attachment site for retinal in the above proteins is a conserved lysine residue in the middle of the seventh transmembrane helix. The pattern that had been developed includes this residue.

-Consensus pattern: [LIVMWAC]-[PGC]-x(3)-[SAC]-K-[STALIMR]-[GSACPNV]-[STACP]-
x(2)-[DENF]-[AP]-x(2)-[IY]
[K is the retinal binding site]

[1] Applebury M.L., Hargrave P.A.
Vision Res. 26:1881-1895(1986).
[2] Fryxell K.J., Meyerowitz E.M.
J. Mol. Evol. 33:367-378(1991).
[3] Shen D., Jiang M., Hao W., Tao L., Salazar M., Fong H.K.W.
Biochemistry 33:13117-13125(1994).

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The following descriptions of protein family functions are not provided by the Pfam or Prosite databases.

740. BAH

BAH domain. Number of members: 65

[1] Medline: 97074677. Molecular cloning of polybromo, a nuclear protein containing multiple domains including five bromodomains, a truncated HMG-box, and two repeats of a novel domain. Nicolas RH, Goodwin GH; Gene 1996;175:233-240.

[2] Medline: 99198739. The BAH (bromo-adjacent homology) domain: a link between DNA methylation, replication and transcriptional regulation. Callebaut I, Courvalin J-C, Mornon JP; FEBS letts 1999;446:189-193.

741. ELM2.

ELM2 domain. The ELM2 (Egl-27 and MTA1 homology 2) domain is a small domain of unknown function. Number of members: 10

742. Euk proin. EUKARYOTIC_PORIN The major protein of the outer mitochondrial membrane of eukaryotes is a porin that forms a voltage-dependent anion-selective channel (VDAC) that behaves as a general diffusion pore for small hydrophilic molecules [1 to 4]. The channel adopts an open conformation at low or zero membrane potential and a closed conformation at potentials above 30-40 mV.

This protein contains about 280 amino acids and its sequence is composed of between 12 to 16 beta-strands that span the mitochondrial outer membrane. Yeast contains two members of this family (genes POR1 and POR2); vertebrates have at least three members (genes VDAC1, VDAC2 and VDAC3) [5].

A conserved region located at the C-terminal part of these proteins was selected as a signature pattern.

Consensus pattern[YH]-x(2)-D-[SPCAD]-x-[STA]-x(3)-[TAG]-[KR]-[LIVMF]-[DNSTA]-[DNS]-x(4)-[GSTAN]-[LIVMA]-x-[LIVMY]

[1] Benz R. Biochim. Biophys. Acta 1197:167-196(1994).
[2] Manella C.A. Trends Biochem. Sci. 17:315-320(1992).
[3] Dihanich M. Experientia 46:146-153(1990).
[4] Forte M., Guy H.R., Mannella C.A. J. Bioenerg. Biomembr. 19:341-350(1987).
[5] Sampson M.J., Lovell R.S., Davison D.B., Craigen W.J. Genomics 36:192-196(1996).

743. Glyco hydor 19

Chitinases family 19 signatures

cross-reference(s) CHITINASE_19_1, CHITINASE_19_2

Chitinases (EC 3.2.1.14) [1] are enzymes that catalyze the hydrolysis of the beta-1,4-N-acetyl-D-glucosamine linkages in chitin polymers. From the view point of sequence similarity chitinases belong to either family 18 or 19 in the classification of glycosyl hydrolases [2,E1]. Chitinases of family 19 (also known as classes IA or I and IB or II) are enzymes from plants that function in the defense against fungal and insect pathogens by destroying their chitin-containing cell wall. Class IA/I and IB/II enzymes differ in the presence (IA/I) or absence (IB/II) of a N-terminal chitin-binding domain (see the relevant entry <PDOC00025>). The catalytic domain of these enzymes consist of about 220 to 230 amino acid residues.

Two highly conserved regions were selected as signature patterns, the first one is located in the N-terminal section and contains one of the six cysteines which are conserved in most, if not all, of these chitinases and which is probably involved in a disulfide bond.

Consensus patternC-x(4,5)-F-Y-[ST]-x(3)-[FY]-[LIVMF]-x-A-x(3)-[YF]-x(2)-F-[GSA]
Consensus pattern[LIVM]-[GSA]-F-x-[STAG](2)-[LIVMFY]-W-[FY]-W-[LIVM]

[1]Flach J., Pilet P.-E., Jolles P. Experientia 48:701-716(1992).
[2] Henrissat B. Biochem. J. 280:309-316(1991).

744. MBD

Methyl-CpG binding domain

The Methyl-CpG binding domain (MBD) binds to DNA that contains one or more symmetrically methylated CpGs [1]. DNA methylation in animals is associated with alterations in chromatin structure and silencing of gene expression. MBD has negligible non-specific affinity for DNA. In vitro foot-printing with MeCP2 showed the MBD can protect a 12 nucleotide region surrounding a methyl CpG pair [1]. MBDs are found in several Methyl-CpG binding proteins and also DNA demethylase [2]. Number of members: 11

[1]Medline: 94232813. Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. Nan X, Meehan RR, Bird A; Nucleic Acids Res 1993;21:4886-4892.
[2]Medline: 99158138. A mammalian protein with specific demethylase activity for mCpG DNA. Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M; Nature 1999;397:579-583.

745. Peptidase C1

Eukaryotic thiol (cysteine) proteases active sites

cross-reference(s) THIOL_PROTEASE_CYS; THIOL_PROTEASE_HIS; THIOL_PROTEASE_ASN

Eukaryotic thiol proteases (EC 3.4.22.-) [1] are a family of proteolytic enzymes which contain an active site cysteine. Catalysis proceeds through a thioester intermediate and is facilitated by a nearby histidine side chain; an asparagine completes the essential catalytic triad. The proteases which are currently known to belong to this family are listed below (references are only provided for recently determined sequences).

- Vertebrate lysosomal cathepsins B (EC 3.4.22.1), H (EC 3.4.22.16), L (EC 3.4.22.15), and S (EC 3.4.22.27) [2].
- Vertebrate lysosomal dipeptidyl peptidase I (EC 3.4.14.1) (also known as cathepsin C) [2].
- Vertebrate calpains (EC 3.4.22.17). Calpains are intracellular calcium- activated thiol protease that contain both a N-terminal catalytic domain and a C-terminal calcium-binding domain.
- Mammalian cathepsin K, which seems involved in osteoclastic bone resorption [3].
- Human cathepsin O [4].
- Bleomycin hydrolase. An enzyme that catalyzes the inactivation of the antitumor drug BLM (a glycopeptide).
- Plant enzymes: barley aleurain (EC 3.4.22.16), EP-B1/B4; kidney bean EP-C1, rice bean SH-EP; kiwi fruit actinidin (EC 3.4.22.14); papaya latex papain (EC 3.4.22.2), chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30), and proteinase IV (EC 3.4.22.25); pea turgor-responsive protein 15A; pineapple stem bromelain (EC 3.4.22.32); rape COT44; rice oryzain alpha, beta, and gamma; tomato low-temperature induced, Arabidopsis thaliana A494, RD19A and RD21A.
- House-dust mites allergens DerP1 and EurM1.
- Cathepsin B-like proteinases from the worms Caenorhabditis elegans (genes gcp-1, cpr-3, cpr-4, cpr-5 and cpr-6), Schistosoma mansoni (antigen SM31) and Japonica (antigen SJ31), Haemonchus contortus (genes AC-1 and AC-2), and Ostertagia ostertagi (CP-1 and CP-3).

- Slime mold cysteine proteinases CP1 and CP2.
- Cruzipain from Trypanosoma cruzi and brucei.
- Throphozoite cysteine proteinase (TCP) from various Plasmodium species.
- Proteases from Leishmania mexicana, Theileria annulata and Theileria parva.
- Baculoviruses cathepsin-like enzyme (v-cath).
- Drosophila small optic lobes protein (gene sol), a neuronal protein that contains a calpain-like domain.
- Yeast thiol protease BLH1/YCP1/LAP3.
- Caenorhabditis elegans hypothetical protein C06G4.2, a calpain-like protein.

Two bacterial peptidases are also part of this family:

- Aminopeptidase C from Lactococcus lactis (gene pepC) [5].
- Thiol protease tpr from Porphyromonas gingivalis.

Three other proteins are structurally related to this family, but may have lost their proteolytic activity.

- Soybean oil body protein P34. This protein has its active site cysteine replaced by a glycine.
- Rat testin, a sertoli cell secretory protein highly similar to cathepsin L but with the active site cysteine is replaced by a serine. Rat testin should not be confused with mouse testin which is a LIM-domain protein (see <PDOC00382>).
- Plasmodium falciparum serine-repeat protein (SERA), the major blood stage antigen. This protein of 111 Kd possesses a C-terminal thiol-protease-like domain [6], but the active site cysteine is replaced by a serine.

The sequences around the three active site residues are well conserved and can be used as signature patterns.

Consensus patternQ-x(3)-[GE]-x-C-[YW]-x(2)-[STAGC]-[STAGCV] [C is the active site residue]

Note the residue in position 4 of the pattern is almost always cysteine; the only exceptions are calpains (Leu), bleomycin hydrolase (Ser) and yeast YCP1 (Ser). Note the residue in position 5 of the pattern is always Gly except in papaya protease IV where it is Glu.

Consensus pattern[LIVMGSTAN]-x-H-[GSACE]-[LIVM]-x-[LIVMAT](2)-G-x-[GSADNH] [H is the active site residue]

Consensus pattern[FYCH]-[WI]-[LIVT]-x-[KRQAG]-N-[ST]-W-x(3)-[FYW]-G-x(2)-G-[LFYW]-[LIVMFYG]-x-[LIVMF] [N is the active site residue]

Note these proteins belong to family C1 (papain-type) and C2 (calpains) in the classification of peptidases [7,E1].

[1]Dufour E. Biochimie 70:1335-1342(1988).
[2]Kirschke H., Barrett A.J., Rawlings N.D. Protein Prof. 2:1587-1643(1995).
[3]Shi G.-P., Chapman H.A., Bhairi S.M., Deleeuw C., Reddy V.Y., Weiss S.J. FEBS Lett. 357:129-134(1995).
[4]Velasco G., Ferrando A.A., Puente X.S., Sanchez L.M., Lopez-Otin C. J. Biol. Chem. 269:27136-27142(1994).
[5]Chapot-Chartier M.P., Nardi M., Chopin M.C., Chopin A., Gripon J.C. Appl. Environ. Microbiol. 59:330-333(1993).
[6]Higgins D.G., McConnell D.J., Sharp P.M. Nature 340:604-604(1989).
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746. Peptidase M22

Glycoprotease family signature cross-reference(s) GLYCOPROTEASE

Glycoprotease (GCP) (EC 3.4.24.57) [1], or o-syaloglycoprotein endopeptidase, is a metalloprotease secreted by Pasteurella haemolytica which specifically cleaves O-sialoglycoproteins such as glycophorin A. The sequence of GCP is highly similar to the following uncharacterized proteins:

- Escherichia coli hypothetical protein ygjD (ORF-X).
- Bacillus subtilis hypothetical protein ydiE.
- Mycobacterium leprae hypothetical protein U229E.
- Mycobacterium tuberculosis hypothetical protein MtCY78.10.

- Synechocystis strain PCC 6803 hypothetical protein slr0807.
- Methanococcus jannaschii hypothetical protein MJ1130.
- Haloarcula marismortui hypothetical protein in HSH 3'region.
- Yeast hypothetical protein YKR038c.
- Yeast hypothetical protein QRI7.

One of the conserved regions contains two conserved histidines. It is possible that this region is involved in coordinating a metal ion such as zinc.

Consensus pattern[KR]-[GSAT]-x(4)-[FYWLH]-[DQNGK]-x-P-x-[LIVMFY]-x(3)-H-x(2)-[AG]-H-[LIVM]
Note these proteins belong to family M22 in the classification of peptidases [2,E1].

[1]Abdullah K.M., Lo R.Y.C., Mellors A. J. Bacteriol. 173:5597-5603(1991).
[2]Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

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747. SAM. SAM domain (Sterile alpha motif)
It has been suggested that SAM is an evolutionarily conserved protein binding domain that is involved in the regulation of numerous developmental processes in diverse eukaryotes. The SAM domain can potentially function as a protein interaction module through its ability to homo- and heterooligomerise with other SAM domains. Number of members: 81

[1]Medline: 96100659 SAM: A novel motif in yeast sterile alpha and Drosophila polyhomeotic proteins Ponting CP; Prot Sci 1995;4:1928-1930.
[2]Medline: 97160498 SAM as a protein interaction domain involved in developmental regulation. Shultz J, Ponting CP, Hofmann K, Bork P; Prot Sci 1997;6:249-253.
[3]Medline: 99101382 The crystal structure of an Eph receptor SAM domain reveals a mechanism for modular dimerization. Reference Author: Stapleton D, Balan I, Pawson T, Sicheri F; Nat Struct Biol 1999;6:44-49.

748. Tyrosinase signatures cross-reference(s) TYROSINASE_1; TYROSINASE_2 Tyrosinase (EC 1.14.18.1) [1] is a copper monooxygenases that catalyzes the hydroxylation of monophenols and the oxidation of o-diphenols to o-quinols. This enzyme, found in prokaryotes as well as in eukaryotes, is involved in the formation of pigments such as melanins and other polyphenolic compounds.

Tyrosinase binds two copper ions (CuA and CuB). Each of the two copper ion has been shown [2] to be bound by three conserved histidines residues. The regions around these copper-binding ligands are well conserved and also shared by some hemocyanins, which are copper-containing oxygen carriers from the hemolymph of many molluscs and arthropods [3,4].

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At least two proteins related to tyrosinase are known to exist in mammals:

- TRP-1 (TYRP1) [5], which is responsible for the conversion of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to indole-5,6-quinone-2-carboxylic acid.
- TRP-2 (TYRP2) [6], which is the melanogenic enzyme DOPAchrome tautomerase (EC 5.3.3.12) that catalyzes the conversion of DOPAchrome to DHICA. TRP-2 differs from tyrosinases and TRP-1 in that it binds two zinc ions instead of copper [7].

Other proteins that belong to this family are:

- Plants polyphenol oxidases (PPO) (EC 1.10.3.1) which catalyze the oxidation of mono- and o-diphenols to o-diquinones [8].
- Caenorhabditis elegans hypothetical protein C02C2.1.

Two signature patterns for tyrosinase and related proteins have been derived The first one contains two of the histidines that bind CuA, and is located in the N-terminal section of tyrosinase. The second pattern contains a histidine that binds CuB, that pattern is located in the central section of the enzyme.

Consensus pattern H-x(4,5)-F-[LIVMFTP]-x-[FW]-H-R-x(2)-[LM]-x(3)-E

[The two H's are copper ligands]

Consensus patternD-P-x-F-[LIVMFYW]-x(2)-H-x(3)-D [H is a copper ligand]

[1]Lerch K. Prog. Clin. Biol. Res. 256:85-98(1988).
[2]Jackman M.P., Hajnal A., Lerch K. Biochem. J. 274:707-713(1991).
[3]Linzen B. Naturwissenschaften 76:206-211(1989).
[4]Lang W.H., van Holde K.E. Proc. Natl. Acad. Sci. U.S.A. 88:244-248(1991).
[5]Kobayashi T., Urabe K., Winder A., Jimenez-Cervantes C., Imokawa G., Brewington T., Solano F., Garcia-Borron J.C., Hearing V.J. EMBO J. 13:5818-5825(1994).
[6]Jackson I.J., Chambers D.M., Tsukamoto K., Copeland N.G., Gilbert D.J., Jenkins N.A., Hearing V. EMBO J. 11:527-535(1992).
[7]Solano F., Martinez-Liarte J.H., Jimenez-Cervantes C., Garcia-Borron J.C., Lozano J.A. Biochem. Biophys. Res. Commun. 204:1243-1250(1994).
[8]Cary J.W., Lax A.R., Flurkey W.H. Plant Mol. Biol. 20:245-253(1992).

749. (Mur Ligase) Folylpolyglutamate synthase signatures

Folylpolyglutamate synthase (EC 6.3.2.17) (FPGS) [1] is the enzyme of folate metabolism that catalyzes ATP-dependent addition of glutamate moieties to tetrahydrofolate.

Its sequence is moderately conserved between prokaryotes (gene folC) and eukaryotes. We developed two signature patterns based on the conserved regions which are rich in glycine residues and could play a role in the catalytical activity and/or in substrate binding.

Description of pattern(s) and/or profile(s)

Consensus pattern[LIVMFY]-x-[LIVM]-[STAG]-G-T-[NK]-G-K-x-[ST]-x(7)- [LIVM](2)-x(3)-[GSK]

Consensus pattern[LIVMFY](2)-E-x-G-[LIVM]-[GA]-G-x(2)-D-x-[GST]-x-[LIVM](2)

[1]Shane B., Garrow T., Brenner A., Chen L., Choi Y.J., Hsu J.C., Stover P. Adv. Exp. Med. Biol. 338:629-634(1993).

750. (Peptidase M3) Neutral zinc metallopeptidases, zinc-binding region signature

The majority of zinc-dependent metallopeptidases (with the notable exception of the carboxypeptidases) share a common pattern of primary structure [1,2,3] in the part of their sequence involved in the binding of zinc, and can be grouped together as a superfamily,known as the metzincins, on the basis of this sequence similarity. They can be classified into a number of distinct families [4,E1] which are listed below along with the proteases which are currently known to belong to these families.

Family M1

- Bacterial aminopeptidase N (EC 3.4.11.2) (gene pepN).
- Mammalian aminopeptidase N (EC 3.4.11.2).
- Mammalian glutamyl aminopeptidase (EC 3.4.11.7) (aminopeptidase A). It may play a role in regulating growth and differentiation of early B-lineage cells.
- Yeast aminopeptidase yscII (gene APE2).
- Yeast alanine/arginine aminopeptidase (gene AAP1).
- Yeast hypothetical protein YIL137c.
- Leukotriene A-4 hydrolase (EC 3.3.2.6). This enzyme is responsible for the hydrolysis of an epoxide moiety of LTA-4 to form LTB-4; it has been shown that it binds zinc and is capable of peptidase activity.

Family M2

- Angiotensin-converting enzyme (EC 3.4.15.1) (dipeptidyl carboxypeptidase I) (ACE) the enzyme responsible for hydrolyzing angiotensin I to angiotensin II. There are two forms of ACE: a testis-specific isozyme and a somatic isozyme which has two active centers.

Family M3

- Thimet oligopeptidase (EC 3.4.24.15), a mammalian enzyme involved in the cytoplasmic degradation of small peptides.
- Neurolysin (EC 3.4.24.16) (also known as mitochondrial oligopeptidase M or microsomal endopeptidase).

- Mitochondrial intermediate peptidase precursor (EC 3.4.24.59) (MIP). It is involved the second stage of processing of some proteins imported in the mitochondrion.
- Yeast saccharolysin (EC 3.4.24.37) (proteinase yscD).
- Escherichia coli and related bacteria dipeptidyl carboxypeptidase (EC 3.4.15.5) (gene dcp).
- Escherichia coli and related bacteria oligopeptidase A (EC 3.4.24.70) (gene opdA or prlC).
- Yeast hypothetical protein YKL134c.

Family M4

- Thermostable thermolysins (EC 3.4.24.27), and related thermolabile neutral proteases (bacillolysins) (EC 3.4.24.28) from various species of Bacillus.
- Pseudolysin (EC 3.4.24.26) from Pseudomonas aeruginosa (gene lasB).
- Extracellular elastase from Staphylococcus epidermidis.
- Extracellular protease prt1 from Erwinia carotovora.
- Extracellular minor protease smp from Serratia marcescens.
- Vibriolysin (EC 3.4.24.25) from various species of Vibrio.
- Protease prtA from Listeria monocytogenes.
- Extracellular proteinase proA from Legionella pneumophila.

Family M5

- Mycolysin (EC 3.4.24.31) from Streptomyces cacaoi.

Family M6

- Immune inhibitor A from Bacillus thuringiensis (gene ina). Ina degrades two classes of insect antibacterial proteins, attacins and cecropins.

Family M7

- Streptomyces extracellular small neutral proteases

Family M8

- Leishmanolysin (EC 3.4.24.36) (surface glycoprotein gp63), a cell surface protease from various species of Leishmania.

Family M9

- Microbial collagenase (EC 3.4.24.3) from Clostridium perfringens and Vibrio alginolyticus.

Family M10A

- Serralysin (EC 3.4.24.40), an extracellular metalloprotease from Serratia.
- Alkaline metalloproteinase from Pseudomonas aeruginosa (gene aprA).
- Secreted proteases A, B, C and G from Erwinia chrysanthemi.
- Yeast hypothetical protein YIL108w.

Family M10B

- Mammalian extracellular matrix metalloproteinases (known as matrixins) [5]: MMP-1 (EC 3.4.24.7) (interstitial collagenase), MMP-2 (EC 3.4.24.24) (72 Kd gelatinase), MMP-9 (EC 3.4.24.35) (92 Kd gelatinase), MMP-7 (EC 3.4.24.23) (matrylisin), MMP-8 (EC 3.4.24.34) (neutrophil collagenase), MMP-3 (EC 3.4.24.17) (stromelysin-1), MMP-10 (EC 3.4.24.22) (stromelysin-2), and MMP-11 (stromelysin-3), MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).
- Sea urchin hatching enzyme (envelysin) (EC 3.4.24.12). A protease that allows the embryo to digest the protective envelope derived from the egg extracellular matrix.
- Soybean metalloendoproteinase 1.

Family M11

- Chlamydomonas reinhardtii gamete lytic enzyme (GLE).

Family M12A

- Astacin (EC 3.4.24.21), a crayfish endoprotease.
- Meprin A (EC 3.4.24.18), a mammalian kidney and intestinal brush border metalloendopeptidase.
- Bone morphogenic protein 1 (BMP-1), a protein which induces cartilage and bone formation and which expresses metalloendopeptidase activity. The Drosophila homolog of BMP-1 is the dorsal-ventral patterning protein tolloid.
- Blastula protease 10 (BP10) from Paracentrotus lividus and the related protein SpAN from Strongylocentrotus purpuratus.

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- Caenorhabditis elegans protein toh-2.
- Caenorhabditis elegans hypothetical protein F42A10.8.
- Choriolysins L and H (EC 3.4.24.67) (also known as embryonic hatching proteins LCE and HCE) from the fish Oryzias lapides. These proteases participates in the breakdown of the egg envelope, which is derived from the egg extracellular matrix, at the time of hatching.

Family M12B

- Snake venom metalloproteinases [6]. This subfamily mostly groups proteases that act in hemorrhage. Examples are: adamalysin II (EC 3.4.24.46), atrolysin C/D (EC 3.4.24.42), atrolysin E (EC 3.4.24.44), fibrolase (EC 3.4.24.72), trimerelysin I (EC 3.4.25.52) and II (EC 3.4.25.53).
- Mouse cell surface antigen MS2.

Family M13

- Mammalian neprilysin (EC 3.4.24.11) (neutral endopeptidase) (NEP).
- Endothelin-converting enzyme 1 (EC 3.4.24.71) (ECE-1), which process the precursor of endothelin to release the active peptide.
- Kell blood group glycoprotein, a major antigenic protein of erythrocytes. The Kell protein is very probably a zinc endopeptidase.
- Peptidase O from Lactococcus lactis (gene pepO).

Family M27

- Clostridial neurotoxins, including tetanus toxin (TeTx) and the various botulinum toxins (BoNT). These toxins are zinc proteases that block neurotransmitter release by proteolytic cleavage of synaptic proteins such as synaptobrevins, syntaxin and SNAP-25 [7,8].

Family M30

- Staphylococcus hyicus neutral metalloprotease.

Family M32

- Thermostable carboxypeptidase 1 (EC 3.4.17.19) (carboxypeptidase Taq), an enzyme from Thermus aquaticus which is most active at high temperature.

Family M34

- Lethal factor (LF) from Bacillus anthracis, one of the three proteins composing the anthrax toxin.

Family M35

- Deuterolysin (EC 3.4.24.39) from Penicillium citrinum and related proteases from various species of Aspergillus.

Family M36

- Extracellular elastinolytic metalloproteinases from Aspergillus.

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From the tertiary structure of thermolysin, the position of the residues acting as zinc ligands and those involved in the catalytic activity are known. Two of the zinc ligands are histidines which are very close together in the sequence; C-terminal to the first histidine is a glutamic acid residue which acts as a nucleophile and promotes the attack of a water molecule on the carbonyl carbon of the substrate. A signature pattern which includes the two histidine and the glutamic acid residues is sufficient to detect this superfamily of proteins.

Description of pattern(s) and/or profile(s)
Consensus pattern[GSTALIVN]-x(2)-H-E-[LIVMFYW]-{DEHRKP}-H-x-
[LIVMFYWGSPQ] [The
two H's are zinc ligands] [E is the active site residue]
Sequences known to belong to this class detected by the patternALL,
except for members of families M5, M7 amd M11.
Other sequence(s) detected in SWISS-PROT55; including Neurospora
crassa conidiation-specific protein 13 which could be a
zinc-protease.
[1]Jongeneel C.V., Bouvier J., Bairoch A.
FEBS Lett. 242:211-214(1989).

[2]Murphy G.J.P., Murphy G., Reynolds J.J.
FEBS Lett. 289:4-7(1991).
[3]Bode W., Grams F., Reinemer P., Gomis-Rueth F.-X., Baumann U., McKay D.B., Stoecker W.
Zoology 99:237-246(1996).
[4]Rawlings N.D., Barrett A.J.
Meth. Enzymol. 248:183-228(1995).
[5]Woessner J. Jr.
FASEB J. 5:2145-2154(1991).
[6]Hite L.A., Fox J.W., Bjarnason J.B.
[7]Montecucco C., Schiavo G.
Trends Biochem. Sci. 18:324-327(1993).
[8]Niemann H., Blasi J., Jahn R.
Trends Cell Biol. 4:179-185(1994).

09655226.061801

751. PseudoU_synt_1

tRNA pseudouridine synthase is involved in the formation of pseudouridine at the anticodon stem and loop of transfer-RNAs Pseudouridine is an isomer of uridine (5-(beta-D-ribofuranosyl) uracil, and id the most abundant modified nucleoside found in all cellular RNAs. The TruA-like proteins also exhibit a conserved sequence with a strictly conserved aspartic acid, likely involved in catalysis. Number of members: 25

[1]Medline: 98254513. Transfer RNA-pseudouridine synthetase Pus1 of Saccaromyces cerevisiae contains one atom of zinc essential for its native conformation and tRNA recognition. Arluison V, Hountondji C, Robert B, Grosjean H; Biochemistry 1998;37:7268-7276.

752. EPSP synthase signatures

EPSP synthase (3-phosphoshikimate 1-carboxyvinyltransferase) (EC 2.5.1.19) catalyzes the sixth step in the biosynthesis from chorismate of the aromatic amino acids (the shikimate pathway) in bacteria (gene aroA), plants and fungi (where it is part of a multifunctional enzyme which catalyzes five consecutive steps in this pathway) [1]. EPSP synthase has been extensively studied as it is the target of the potent herbicide glyphosate which inhibits the enzyme.

The sequence of EPSP from various biological sources shows that the structure of the enzyme has been well conserved throughout evolution. Two conserved regions were selected as signature patterns. The first pattern corresponds to a region that is part of the active site and which is also important for the resistance to glyphosate [2]. The second pattern is located in the C-terminal part of the protein and contains a conserved lysine which seems to be important for the activity of the enzyme.

Description of pattern(s) and/or profile(s)

Consensus pattern[LIVM]-x(2)-[GN]-N-[SA]-G-T-[STA]-x-R-x-[LIVMY]-x-[GSTA]
Consensus pattern[KR]-x-[KH]-E-[CST]-[DNE]-R-[LIVM]-x-[STA]-[LIVMC]-x(2)-[EN]-[LIVMF]-x-[KRA]-[LIVMF]-G

[1]Stallings W.C., Abdel-Megid S.S., Lim L.W., Shieh H.-S., Dayringer H.E., Leimgruber N.K., Stegeman R.A., Anderson K.S., Sikorski J.A., Padgette S.R., Kishore G.M. Proc. Natl. Acad. Sci. U.S.A. 88:5046-5050(1991).
[2]Padgette S.R., Re D.B., Gaser C.S., Eicholtz D.A., Frazier R.B., Hironaka C.M., Levine E.B., Shah D.M., Fraley R.T., Kishore G.M. J. Biol. Chem. 266:22364-22369(1991).

753. Glyco_hydro_18
Glycosyl hydrolases family 18. Number of members: 173
[1]Medline: 95219379. Crystal structure of a bacterial chitinase at 2.3 A resolution. Perrakis A, Tews I, Dauter Z, Oppenheim AB, Chet I, Wilson KS, Vorgias CE; Structure 1994;2:1169-1180.

754. Esterase
Putative esterase

This family contains Esterase D Swiss:P10768. However it is not clear if all members of the family have the same function. This family is possibly related to the COesterase family.

Number of members: 36

755. (HMA) Heavy-metal-associated domain

A conserved domain of about 30 amino acid residues has been found [1] in a number of proteins that transport or detoxify heavy metals. This domain contains two conserved cysteines that could be involved in the binding of these metals. The domain has been termed Heavy-Metal-Associated (HMA). It has been found in:

- A variety of cation transport ATPases (E1-E2 ATPases) (see <PDOC00139>). The human copper ATPAses ATP7A and ATP7B which are respectively involved in Menke's and Wilson's diseases. ATP7A and ATP7B both contain 6 tandem copies of the HMA domain. The copper ATPases CCC2 from budding yeast, copA from Enterococcus faecalis and synA from Synechococcus contain one copy of the HMA domain. The cadmium ATPases cadA from Bacillus firmus and from plasmid pI258 from Staphylococcus aureus also contain a single HMA domain, while a chromosomal Staphylococcus aureus cadA contains two copies. Other, less characterized ATPases that contain the HMA domain are: fixI from Rhizobium meliloti, pacS from Synechococcus strain PCC 7942), Mycobacterium leprae ctpA and ctpB and Escherichia coli hypothetical protein yhhO. In all these ATPases the HMA domain(s) are located in the N-terminal section.
- Mercuric reductase (EC 1.16.1.1) (gene merA) which is generally encoded by plasmids carried by mercury-resistant Gram-negative bacteria. Mercuric reductase is a class-1 pyridine nucleotide-disulphide oxidoreductase (see <PDOC00073>). There is generally one HMA domain (with the exception of a chromosomal merA from Bacillus strain RC607 which has two) in the N-terminal part of merA.
- Mercuric transport protein periplasmic component (gene merP), also encoded by plasmids carried by mercury-resistant Gram-negative bacteria. It seems to be a mercury scavenger that specifically binds to one Hg(2+) ion and which passes it to the mercuric reductase via the merT protein. The N-terminal half of merP is a HMA domain.
- Helicobacter pylori copper-binding protein copP.

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- Yeast protein ATX1 [2], which could act in the transport and/or partitioning of copper.

The consensus pattern for HMA spans the complete domain.

Description of pattern(s) and/or profile(s)

Consensus pattern[LIVN]-x(2)-[LIVMFA]-x-C-x-[STAGCDNH]-C-x(3)-[LIVFG]-x(3)-[LIV]-x(9,11)-[IVA]-x-[LVFYS] [The two C's probably bind metals]

[1]Bull P.C., Cox D.W. Trends Genet. 10:246-252(1994).
[2]Lin S.-J., Culotta V.L. Proc. Natl. Acad. Sci. U.S.A. 92:3784-3788(1995).

756. (Peptidase M10) Matrixins cysteine switch

PROSITE cross-reference(s): CYSTEINE_SWITCH

Mammalian extracellular matrix metalloproteinases (EC 3.4.24.-), also known as matrixins [1] (see <PDOC00129>), are zinc-dependent enzymes. They are secreted by cells in an inactive form (zymogen) that differs from the mature enzyme by the presence of an N-terminal propeptide. A highly conserved octapeptide is found two residues downstream of the C-terminal end of the propeptide. This region has been shown to be involved in autoinhibition of matrixins [2,3]; a cysteine within the octapeptide chelates the active site zinc ion, thus inhibiting the enzyme. This region has been called the 'cysteine switch' or 'autoinhibitor region'.

A cysteine switch has been found in the following zinc proteases:

- MMP-1 (EC 3.4.24.7) (interstitial collagenase).
- MMP-2 (EC 3.4.24.24) (72 Kd gelatinase).
- MMP-3 (EC 3.4.24.17) (stromelysin-1).
- MMP-7 (EC 3.4.24.23) (matrilysin).
- MMP-8 (EC 3.4.24.34) (neutrophil collagenase).
- MMP-9 (EC 3.4.24.35) (92 Kd gelatinase).
- MMP-10 (EC 3.4.24.22) (stromelysin-2).
- MMP-11 (EC 3.4.24.-) (stromelysin-3).
- MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).

- MMP-13 (EC 3.4.24.-) (collagenase 3).
- MMP-14 (EC 3.4.24.-) (membrane-type matrix metalliproteinase 1).
- MMP-15 (EC 3.4.24.-) (membrane-type matrix metalliproteinase 2).
- MMP-16 (EC 3.4.24.-) (membrane-type matrix metalliproteinase 3).
- Sea urchin hatching enzyme (EC 3.4.24.12) (envelysin) [4].
- Chlamydomonas reinhardtii gamete lytic enzyme (GLE) [5].

Description of pattern(s) and/or profile(s)

Consensus patternP-R-C-[GN]-x-P-[DR]-[LIVSAPKQ] [C chelates the zinc ion]

[1]Woessner J. Jr. FASEB J. 5:2145-2154(1991).

[2]Sanchez-Lopez R., Nicholson R., Gesnel M.C., Matrisian L.M., Breathnach R. J. Biol. Chem. 263:11892-11899(1988).

[3]Park A.J., Matrisian L.M., Kells A.F., Pearson R., Yuan Z., Navre M. J. Biol. Chem. 266:1584-1590(1991).

[4]Lepage T., Gache C. EMBO J. 9:3003-3012(1990).

[5]Kinoshita T., Fukuzawa H., Shimada T., Saito T., Matsuda Y. Proc. Natl. Acad. Sci. U.S.A. 89:4693-4697(1992).

757. (Peptidase S8) Serine proteases, subtilase family, active sites

PROSITE cross-reference(s): PS00136; SUBTILASE_ASP, PS00137; SUBTILASE_HIS, PS00138; SUBTILASE_SER

Subtilases [1,2] are an extensive family of serine proteases whose catalytic activity is provided by a charge relay system similar to that of the trypsin family of serine proteases but which evolved by independent convergent evolution. The sequence around the residues involved in the catalytic triad (aspartic acid, serine and histidine) are completely different from that of the analogous residues in the trypsin serine proteases and can be used as signatures specific to that category of proteases.

The subtilase family currently includes the following proteases:

- Subtilisins (EC 3.4.21.62), these alkaline proteases from various Bacillus species have been the target of numerous studies in the past thirty years.
- Alkaline elastase YaB from Bacillus sp. (gene ale).

- Alkaline serine exoprotease A from Vibrio alginolyticus (gene proA).
- Aqualysin I from Thermus aquaticus (gene pstI).
- AspA from Aeromonas salmonicida.
- Bacillopeptidase F (esterase) from Bacillus subtilis (gene bpf).
- C5A peptidase from Streptococcus pyogenes (gene scpA).
- Cell envelope-located proteases PI, PII, and PIII from Lactococcus lactis.
- Extracellular serine protease from Serratia marcescens.
- Extracellular protease from Xanthomonas campestris.
- Intracellular serine protease (ISP) from various Bacillus.
- Minor extracellular serine protease epr from Bacillus subtilis (gene epr).
- Minor extracellular serine protease vpr from Bacillus subtilis (gene vpr).
- Nisin leader peptide processing protease nisP from Lactococcus lactis.
- Serotype-specific antigene 1 from Pasteurella haemolytica (gene ssa1).
- Thermitase (EC 3.4.21.66) from Thermoactinomyces vulgaris.
- Calcium-dependent protease from Anabaena variabilis (gene prcA).
- Halolysin from halophilic bacteria sp. 172p1 (gene hly).
- Alkaline extracellular protease (AEP) from Yarrowia lipolytica (gene xpr2).
- Alkaline proteinase from Cephalosporium acremonium (gene alp).
- Cerevisin (EC 3.4.21.48) (vacuolar protease B) from yeast (gene PRB1).
- Cuticle-degrading protease (pr1) from Metarhizium anisopliae.
- KEX-1 protease from Kluyveromyces lactis.
- Kexin (EC 3.4.21.61) from yeast (gene KEX-2).
- Oryzin (EC 3.4.21.63) (alkaline proteinase) from Aspergillus (gene alp).
- Proteinase K (EC 3.4.21.64) from Tritirachium album (gene proK).
- Proteinase R from Tritirachium album (gene proR).
- Proteinase T from Tritirachium album (gene proT).
- Subtilisin-like protease III from yeast (gene YSP3).
- Thermomycolin (EC 3.4.21.65) from Malbranchea sulfurea.
- Furin (EC 3.4.21.85), neuroendocrine convertases 1 to 3 (NEC-1 to -3) and PACE4 protease from mammals, other vertebrates, and invertebrates. These proteases are involved in the processing of hormone precursors at sites comprised of pairs of basic amino acid residues [3].
- Tripeptidyl-peptidase II (EC 3.4.14.10) (tripeptidyl aminopeptidase) from Human.

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- Prestalk-specific proteins tagB and tagC from slime mold [4]. Both proteins consist of two domains: a N-terminal subtilase catalytic domain and a C-terminal ABC transporter domain (see <PDOC00185>).

Description of pattern(s) and/or profile(s)

Consensus pattern[STAIV]-x-[LIVMF]-[LIVM]-D-[DSTA]-G-[LIVMFC]-x(2,3)-[DNH] [D is the active site residue]

Consensus patternH-G-[STM]-x-[VIC]-[STAGC]-[GS]-x-[LIVMA]-[STAGCLV]-[SAGM] [H is the active site residue]

Consensus patternG-T-S-x-[SA]-x-P-x(2)-[STAVC]-[AG] [S is the active site residue]

Note if a protein includes at least two of the three active site signatures, the probability of it being a serine protease from the subtilase family is 100%

Note these proteins belong to family S8 in the classification of peptidases [5,E1].

[1]Siezen R.J., de Vos W.M., Leunissen J.A.M., Dijkstra B.W. Protein Eng. 4:719-737(1991).

[2]Siezen R.J. (In) Proceeding subtilisin symposium, Hamburg, (1992).

[3]Barr P.J. Cell 66:1-3(1991).

[4]Shaulsky G., Kuspa A., Loomis W.F.; Genes Dev. 9:1111-1122(1995).

[5]Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

758. (SSB) Single-strand binding protein family signatures

PROSITE cross-reference(s): PS00735; SSB_1,PS00736; SSB_2

The Escherichia coli single-strand binding protein [1] (gene ssb), also known as the helix-destabilizing protein, is a protein of 177 amino acids. It binds tightly, as a homotetramer, to single-stranded DNA (ss-DNA) and plays an important role in DNA replication, recombination and repair.

Closely related variants of SSB are encoded in the genome of a variety of large self-transmissible plasmids. SSB has also been characterized in bacteria such as Proteus mirabilis or Serratia marcescens.

Eukaryotic mitochondrial proteins that bind ss-DNA and are probably involved in mitochondrial DNA replication are structurally and evolutionary related to prokaryotic SSB. Proteins currently known to belong to this subfamily are listed below [2].

- Mammalian protein Mt-SSB (P16).
- Xenopus Mt-SSBs and Mt-SSBr.
- Drosophila MtSSB.
- Yeast protein RIM1.

Two signature patterns have been developed for these proteins. The first is a conserved region in the N-terminal section of the SSB's. The second is a centrally located region which, in Escherichia coli SSB, is known to be involved in the binding of DNA.

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Description of pattern(s) and/or profile(s)

Consensus pattern[LIVMF]-[NST]-[KRT]-[LIVM]-x-[LIVMF](2)-G-[NHRK]-[LIVM]-[GST]-x-[DET]

Consensus patternT-x-W-[HY]-[RNS]-[LIVM]-x-[LIVMF]-[FY]-[NGKR]

[1]Meyer R.R., Laine P.S. Microbiol. Rev. 54:342-380(1990).
[2]Stroumbakis N.D., Li Z., Tolias P.P. Gene 143:171-177(1994).

759. KDPG and KHG aldolases active site signatures

PROSITE cross-reference(s): PS00159; ALDOLASE_KDPG_KHG_1, PS00160; ALDOLASE_KDPG_KHG_2

4-hydroxy-2-oxoglutarate aldolase (EC 4.1.3.16) (KHG-aldolase) catalyzes the interconversion of 4-hydroxy-2-oxoglutarate into pyruvate and glyoxylate. Phospho-2-dehydro-3-deoxygluconate aldolase (EC 4.1.2.14) (KDPG-aldolase) catalyzes the interconversion of 6-phospho-2-dehydro-3-deoxy-D-gluconate into pyruvate and glyceraldehyde 3-phosphate.

These two enzymes are structurally and functionally related [1]. They are both homotrimeric proteins of approximately 220 amino-acid residues. They are class I aldolases whose catalytic mechanism involves the formation of a Schiff-base intermediate between the substrate and the epsilon-amino group of a lysine residue. In both enzymes, an arginine is required for catalytic activity.

Two signature patterns were developed for these enzymes. The first one contains the active site arginine and the second, the lysine involved in the Schiff-base formation.

Description of pattern(s) and/or profile(s)
Consensus patternG-[LIVM]-x(3)-E-[LIV]-T-[LF]-R [R is the active site residue]
Consensus patternG-x(3)-[LIVMF]-K-[LF]-F-P-[SA]-x(3)-G [K is involved in Schiff-base formation]

[1] Vlahos C J., Dekker E.E. J. Biol. Chem. 263:11683-11691(1988).

760. AP endonucleases family 1 signatures. PROSITE cross-reference(s): PS00726; AP_NUCLEASE_F1_1, PS00727; AP_NUCLEASE_F1_2, PS00728; AP_NUCLEASE_F1_3

DNA damaging agents such as the antitumor drugs bleomycin and neocarzinostatin or those that generate oxygen radicals produce a variety of lesions in DNA. Amongst these is base-loss which forms apurinic/apyrimidinic (AP) sites or strand breaks with atypical 3'termini. DNA repair at the AP sites is initiated by specific endonuclease cleavage of the phosphodiester backbone. Such endonucleases are also generally capable of removing blocking groups from the 3'terminus of DNA strand breaks.

AP endonucleases can be classified into two families on the basis of sequence similarity. Family 1 groups the enzymes listed below [1].

- Escherichia coli exonuclease III (EC 3.1.11.2) (gene xthA).
- Streptococcus pneumoniae and Bacillus subtilis exonuclease A (gene exoA).
- Mammalian AP endonuclease 1 (AP1) (EC 4.2.99.18).
- Drosophila recombination repair protein 1 (gene Rrp1).
- Arabidopsis thaliana apurinic endonuclease-redox protein (gene arp).

Except for Rrp1 and arp, these enzymes are proteins of about 300 amino-acid residues. Rrp1 and arp both contain additional and unrelated sequences in their N-terminal section (about 400 residues for Rrp1 and 270 for arp).

Three signature patterns were developed for this family of enzymes. The patterns are based on the most conserved regions. The first pattern contains a glutamate which has been shown [2], in the Escherichia coli enzyme to bind a divalent metal ion such as magnesium or manganese

Consensus pattern[APF]-D-[LIVMF](2)-x-[LIVM]-Q-E-x-K [E binds a divalent metal ion]
Consensus patternD-[ST]-[FY]-R-[KH]-x(7,8)-[FYW]-[ST]-[FYW](2)
Consensus patternN-x-G-x-R-[LIVM]-D-[LIVMFYH]-x-[LV]-x-S

[1] Barzilay G., Hickson I.S. BioEssays 17:713-719(1995).
[2] Mol C.D., Kuo C.-F., Thayer M.M., Cunningham R.P., Tainer J.A. Nature 374:381-386(1995).

761. (ER)Enhancer of rudimentary signature, PROSITE cross-reference(s): PS01290; ER

The Drosophila protein 'enhancer of rudimentary' (gene (e(r)) is a small protein of 104 residues whose function is not yet clear. From an evolutionary point of view, it is highly conserved [1] and has been found to exist in probably all multicellular eukaryotic organisms. It has been proposed that this protein plays a role in the cell cycle.

A conserved region in the central part of the protein was selected as as signaure pattern.

Consensus patternY-D-I-[SA]-x-L-[FY]-x-F-[IV]-D-x(3)-D-[LIV]-S

[1] Gelsthorpe M., Pulumati M., McCallum C., Dang-Vu K., Tsubota S.I. Gene 186:189-195(1997).

762. (ETF alpha) Electron transfer flavoprotein alpha-subunit signature, PROSITE cross-reference(s): PS00696; ETF_ALPHA

The electron transfer flavoprotein (ETF) [1,2] serves as a specific electron acceptor for various mitochondrial dehydrogenases. ETF transfers electrons to the main respiratory chain via ETF-ubiquinone oxidoreductase. ETF is an heterodimer that consist of an alpha and a beta subunit and which bind one molecule of FAD per dimer. A similar system also exists in some bacteria.

The alpha subunit of ETF is a protein of about 32 Kd which is structurally related to the bacterial nitrogen fixation protein fixB which could play a role in a redox process and feed electrons to ferredoxin.

Other related proteins are:

- Escherichia coli hypothetical protein ydiR.
- Escherichia coli hypothetical protein ygcQ.

A highly conserved region which is located in the C-terminal section was selected as a signature pattern for these proteins.

Consensus pattern [LI]-Y-[LIVM]-[AT]-x-G-[IV]-[SD]-G-x-[IV]-Q-H-x(2)-G-x(6)-[IV]-x-A-[IV]-N

[1] Finocchiaro G., Ikeda Y., Ito M., Tanaka K. Prog. Clin. Biol. Res. 321:637-652(1990).
[2] Tsai M.H., Saier M.H. Jr. Res. Microbiol. 146:397-404(1995).

763. (lectin c) C-type lectin domain signature and profile
PROSITE cross-reference(s): PS00615; C_TYPE_LECTIN_1, PS50041; C_TYPE_LECTIN_2

A number of different families of proteins share a conserved domain which was first characterized in some animal lectins and which seem to function as a calcium-dependent carbohydrate-recognition domain [1,2,3]. This domain, which is known as the C-type lectin domain (CTL) or as the carbohydrate-recognition domain (CRD), consists of about 110 to 130 residues. There are four cysteines which are perfectly conserved and involved in two disulfide bonds. A schematic representation of the CTL domain is shown below.

```
  +------+
  |    |
xcxxxxcxxxxxxxCxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxCxxxxWxCxxxxCx
|  |    |*************|*
+----+    +--------------------------------------------+
```

'C': conserved cysteine involved in a disulfide bond.
'c': optional cysteine involved in a disulfide bond.
'*': position of the pattern.

The categories of proteins, in which the CTL domain has been found, are listed below.

Type-II membrane proteins where the CTL domain is located at the C-terminal extremity of the proteins:

- Asialoglycoprotein receptors (ASGPR) (also known as hepatic lectins) [4]. The ASGPR's mediate the endocytosis of plasma glycoproteins to which the terminal sialic acid residue in their carbohydrate moieties has been removed.
- Low affinity immunoglobulin epsilon Fc receptor (lymphocyte IgE receptor), which plays an essential role in the regulation of IgE production and in the differentiation of B cells.
- Kupffer cell receptor. A receptor with an affinity for galactose and fucose, that could be involved in endocytosis.
- A number of proteins expressed on the surface of natural killer T-cells: NKG2, NKR-P1, YE1/88 (Ly-49), CD69 and on B-cells: CD72, LyB-2. The CTL- domain in these proteins is distantly related to other CTL-domains; it is unclear whether they are likely to bind carbohydrates.

Proteins that consist of an N-terminal collagenous domain followed by a CTL- domain [5], these proteins are sometimes called 'collectins':

- Pulmonary surfactant-associated protein A (SP-A). SP-A is a calcium-dependent protein that binds to surfactant phospholipids and contributes to lower the surface tension at the air-liquid interface in the alveoli of the mammalian lung.
- Pulmonary surfactant-associated protein D (SP-D).
- Conglutinin, a calcium-dependent lectin-like protein which binds to a yeast cell wall extract and to immune complexes through the complement component (iC3b).
- Mannan-binding proteins (MBP) (also known as mannose-binding proteins). MBP's bind mannose and N-acetyl-D-glucosamine in a calcium-dependent manner.
- Bovine collectin-43 (CL-43).

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Selectins (or LEC-CAM) [6,7]. Selectins are cell adhesion molecules implicated in the interaction of leukocytes with platelets or vascular endothelium. Structurally, selectins consist of a long extracellular domain, followed by a transmembrane region and a short cytoplasmic domain. The extracellular domain is itself composed of a CTL-domain, followed by an EGF-like domain and a variable number of SCR/Sushi repeats. Known selectins are:

- Lymph node homing receptor (also known as L-selectin, leukocyte adhesion molecule-1, (LAM-1), leu-8, gp90-mel, or LECAM-1)
- Endothelial leukocyte adhesion molecule 1 (ELAM-1, E-selectin or LECAM-2). The ligand recognized by ELAM-1 is sialyl-Lewis x.
- Granule membrane protein 140 (GMP-140, P-selectin, PADGEM, CD62, or LECAM-3). The ligand recognized by GMP-140 is Lewis x.

Large proteoglycans that contain a CTL-domain followed by one copy of a SCR/ Sushi repeat, in their C-terminal section:

- Aggrecan (cartilage-specific proteoglycan core protein). This proteoglycan is a major component of the extracellular matrix of cartilagenous tissues where it has a role in the resistance to compression.
- Brevican.
- Neurocan.
- Versican (large fibroblast proteoglycan), a large chondroitin sulfate proteoglycan that may play a role in intercellular signalling.

In addition to the CTL and Sushi domains, these proteins also contain, in their N-terminal domain, an Ig-like V-type region, two or four link domains (see <PDOC00955>) and up to two EGF-like repeats.

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Two type-I membrane proteins:

- Mannose receptor from macrophages. This protein mediates the endocytosis of glycoproteins by macrophages in several recognition and uptake processes. Its extracellular section consists of a fibronectin type II domain followed by eight tandem repeats of the CTL domain.
- 180 Kd secretory phospholipase A2 receptor (PLA2-R). A protein whose structure is highly similar to that of the mannose receptor.
- DEC-205 receptor. This protein is used by dendritic cells and thymic epithelial cells to capture and endocytose diverse carbohydrate-binding antigens and direct them to antigen-processing cellular compartiments. DEC-205 extracellular section consists of a fibronectin type II domain followed by ten tandem repeats of the CTL domain.
- Silk moth hemocytin, an humoral lectin which is involved in a self-defence mechanism. It is composed of 2 FA58C domains (see <PDOC00988>), a CTL domain, 2 VWFC domains (see <PDOC00928), and a CTCK (see <PDOC00912>).

Various other proteins that uniquely consist of a CTL domain:

- Invertebrate soluble galactose-binding lectins. A category to which belong a humoral lectin from a flesh fly; echinoidin, a lectin from the coelomic fluid of a sea urchin; BRA-2 and BRA-3, two lectins from the coelomic fluid of a barnacle, a lectin from the tunicate Polyandrocarpa misakiensis and a newt oviduct lectin. The physiological importance of these lectins is not yet known but they may play an important role in defense mechanisms.

- Pancreatic stone protein (PSP) (also known as pancreatic thread protein (PTP), or reg), a protein that might act as an inhibitor of spontaneous calcium carbonate precipitation.
- Pancreatitis associated protein (PAP), a protein that might be involved in the control of bacterial proliferation.
- Tetranectin, a plasma protein that binds to plasminogen and to isolated kringle 4.
- Eosinophil granule major basic protein (MBP), a cytotoxic protein.
- A galactose specific lectin from a rattlesnake.
- Two subunits of a coagulation factor IX/factor X-binding protein (IX/X-bp), a snake venom anticoagulant protein which binds with factors IX and X in the presence of calcium.
- Two subunits of a phospholipase A2 inhibitor from the plasma of a snake (PLI-A and PLI-B).
- A lipopolysaccharide-binding protein (LPS-BP) from the hemolymph of a cockroach [8].
- Sea raven antifreeze protein (AFP) [9].

As a signature pattern for this domain, the C-terminal region with its three conserved cysteines was selected.

Consensus patternC-[LIVMFYATG]-x(5,12)-[WL]-x-[DNSR]-x(2)-C-x(5,6)-[FYWLIVSTA]-[LIVMSTA]-C [The three C's are involved in disulfide bonds]

Note all CTL domains have five Trp residues before the second Cys, with the exception of tunicate lectin and cockroach LPS-BP which have Leu.

Note this documentation entry is linked to both a signature pattern and a profile. As the profile is much more sensitive than the pattern, you should use it if you have access to the necessary software tools to do so.

[1] Drickamer K. J. Biol. Chem. 263:9557-9560(1988).
[2] Drickamer K. Prog. Nucleic Acid Res. Mol. Biol. 45:207-232(1993).
[3] Drickamer K. Curr. Opin. Struct. Biol. 3:393-400(1993).
[4] Spiess M. Biochemistry 29:10009-10018(1990).
[5] Weis W.I., Kahn R., Fourme R., Drickamer K., Hendrickson W.A. Science 254:1608-1615(1991).
[6] Siegelman M. Curr. Biol. 1:125-128(1991).
[7] Lasky L.A. Science 238:964-969(1992).
[8] Jomori T., Natori S. J. Biol. Chem. 266:13318-13323(1991).
[9] Ng N.F.L., Hew C.-L. J. Biol. Chem. 267:16069-16075(1992).

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764. (SRCR) Speract receptor repeated domain signature
PROSITE cross-reference(s): PS00420; SPERACT_RECEPTOR,

The receptor for the sea urchin egg peptide speract is a transmembrane glycoprotein of 500 amino acid residues [1]. Structurally it consists of a large extracellular domain of 450 residues, followed by a transmembrane region and a small cytoplasmic domain of 12 amino acids. The extracellular domain contains four repeats of a 115 amino acids domain. There are 17 positions that are perfectly conserved in the four repeats, among them are six cysteines, six glycines, and three glutamates.

Such a domain is also found, once, in the C-terminal section of mammalian macrophage scavenger receptor type I [2], a membrane glycoproteins implicated in the pathologic deposition of cholesterol in arterial walls during atherogenesis.

The signature pattern that was derived spans part of the N-terminal section of the domain and contains 8 of the 17 conserved residues.

Consensus patternG-x(5)-G-x(2)-E-x(6)-W-G-x(2)-C-x(3)-[FYW]-x(8)-C-x(3)-G

[1] Dangott J.J., Jordan J.E., Bellet R.A., Garbers D.L. Proc. Natl. Acad. Sci. U.S.A. 86:2128-2132(1989).

[2] Freeman M., Ashkenas J., Rees D.J., Kingsley D.M., Copeland N.G., Jenkins N.A., Krieger M. Proc. Natl. Acad. Sci. U.S.A. 87:8810-8814(1990).

765. Bac_surface_Ag

Bacterial surface antigen

This entry includes the following surface antigens; D15 antigen from H.influenzae, OMA87 from P.multocida, OMP85 from N.meningitidis and N.gonorrhoeae. Number of members: 14

[1]Medline: 95255676. The sequencing of the 80-kDa D15 protective surface antigen of Haemophilus influenzae. Flack FS, Loosmore S, Chong P, Thomas WR; Gene 1995;156:97-99.

[2] Medline: 96333354. Cloning, sequencing, expression, and protective capacity of the oma87 gene encoding the Pasteurella multocida 87-kilodalton outer membrane antigen. Ruffolo CG, Adler B; Infect Immun 1996;64:3161-3167.

766. BRCA1 C Terminus (BRCT) domain

The BRCT domain is found predominantly in proteins involved in cell cycle checkpoint functions responsive to DNA damage. It has been suggested that the Retinoblastoma protein contains a divergent BRCT domain, this has not been included in this family. The BRCT domain of XRCC1 forms a homodimer in the crystal structure Medline:99016060. This suggests that pairs of BRCT domains associate as homo- or heterodimers. Number of members: 131

[1] Medline: 96259550. BRCA1 protein products ...Functional motifs... Koonin EV, Altschul SF, Bork P; Nature Genet 1996;13:266-268.

[2] Medline: 97153217. From BRCA1 to RAP1: A widespread BRCT module closely associated with DNA repair Callebaut I, Mornon JP; Febs lett 1997;400:25-30.

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[3] Medline: 97186552. A superfamily of conserved domains in DNA damage responsive cell cycle checkpoint proteins Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV; Faseb J 1997;11:68-76.

[4] Medline: 97402527. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ; Nucleic Acids Res 1997;25:3389-3402.

[5] Medline: 99016060. Structure of an XRCC1 BRCT domain: a new protein-protein interaction module. Zhang X, Morera S, Bates PA, Whitehead PC, Coffer AI, Hainbucher K, Nash RA, Sternberg MJ, Lindahl T, Freemont PS;

767. Kappa casein

Kappa-casein is a mammalian milk protein involved in a number of important physiological processes. In the gut, the ingested protein is split into an insoluble peptide (para kappa-casein) and a soluble hydrophilic glycopeptide (caseinomacropeptide). Caseinomacropeptide is responsible for increased efficiency of digestion, prevention of neonate hypersensitivity to ingested proteins, and inhibition of gastric pathogens. Number of members: 56

[1] Medline: 98072500. Nucleotide sequence evolution at the kappa-casein locus: evidence for positive selection within the family Bovidae. Ward TJ, Honeycutt RL, Derr JN; Genetics 1997;147:1863-1872.

768. Chitinases family 18 active site

PROSITE cross-reference(s) CHITINASE_18

Chitinases (EC 3.2.1.14) [1] are enzymes that catalyze the hydrolysis of the beta-1,4-N-acetyl-D-glucosamine linkages in chitin polymers. From the view point of sequence similarity chitinases belong to either family 18 or 19 in the classification of glycosyl hydrolases [2,E1]. Chitinases of family 18 (also known as classes III or V) groups a variety of proteins:

a) Chitinases from:

- Prokaryotes such as Alteromonas, Bacillus, Serratia, Streptomyces, etc.
- Plants such as Arabidopsis, cucumber, bean, tobacco, etc.
- Fungi such as Aphanocladium, Rhizopus, Saccharomyces, etc.

- Nematode (Brugia malayi).
- Insects (Manduca sexta).
- Baculoviruses (Autographa Californica Nuclear Polyhedrosis virus).

b) Other proteins:

- Hevamine, a rubber tree protein with chitinase and lysozyme activities.
- Kluyveromyces lactis killer toxin alpha subunit, which acts as a chitinase.
- Flavobacterium and Streptomyces endo-beta-N-acetylglucosaminidases (EC 3.2.1.96).
- Mammalian di-N-acetylchitobiase which is involved in the degradation of asparagine-linked glycoproteins.
- Human cartilage glycoprotein Gp-39.
- Jack bean concanavalin B (conB), a protein that has lost its catalytic activity.

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Site directed mutagenesis experiments [3] and crystallographic data [4,5] have shown that a conserved glutamate is involved in the catalytic mechanism and probably acts as a proton donor. This glutamate is at the extremity of the best conserved region in these proteins.

Consensus pattern[LIVMFY]-[DN]-G-[LIVMF]-[DN]-[LIVMF]-[DN]-x-E [E is the active site residue]

[1] Flach J., Pilet P.-E., Jolles P. Experientia 48:701-716(1992).

[2] Henrissat B. Biochem. J. 280:309-316(1991).

[3] Watanabe T., Kohori K., Miyashita K., Fujii T., Sakai H., Uchida M., Tanaka H. J. Biol. Chem. 268:18567-18572(1993).

[4] Perrakis A., Tews I., Dauter Z., Oppenheim A.B., Chet I., Wilson K.S., Vorgias C.E. Structure 2:1169-1180(1994).

[5] van Scheltinga A.C.T., Kalk K.H., Beintema J.J., Dijkstra B.W. Structure 2:1181-1189(1994).

769. gag_p17. gag gene protein p17 (matrix protein).

The matrix protein forms an icosahedral shell associated with the inner membrane of the mature immunodeficiency virus. Number of members: 1598

[1] Medline: 95055757. Three-dimensional structure of the human immunodeficiency virus type 1 matrix protein. Massiah MA, Starich MR, Paschall C, Summers MF, Christensen AM, Sundquist WI; J Mol Biol 1994;244:198-223.

770. GDA1/CD39 family of nucleoside phosphatases signature
PROSITE cross-reference(s); GDA1_CD39_NTPASE
A number of nucleoside diphosphate and triphosphate hydrolases as well as some yet uncharacterized proteins have been found to belong to the same family [1, 2]. This family currently consist of:

- Yeast guanosine-diphosphatase (EC 3.6.1.42) (GDPase) (gene GDA1). GDA1 is a golgi integral membrane enzyme that catalyzes the hydrolysis of GDP to GMP.
- Potato apyrase (EC 3.6.1.5) (adenosine diphosphatase) (ADPase). Apyrase acts on both ATP and ADP to produce AMP.
- Mammalian vascular ATP-diphosphohydrolase (EC 3.6.1.5) (also known as lymphoid cell activation antigen CD39).
- Toxoplasma gondii nucleoside-triphosphatases (EC 3.6.1.15) (NTPase). NTPase hydrolyses various nucleoside triphosphates to produce the corresponding nucleoside mono- and diphosphates. This enzyme is secreted into the invaded host cell into the parasitophorous vacuole, a specialized compartment where the parasite intracellulary resides.
- Pea nucleoside-triphosphatases (EC 3.6.1.15) (NTPase).

- Caenorhabditis elegans hypothetical protein C33H5.14.
- Caenorhabditis elegans hypothetical protein R07E4.4.
- Yeast chromosome V hypothetical protein YER005w.

The above uncharacterized proteins all seem to be membrane-bound.

All these proteins share a number of conserved domains. The best conserved of these domains have been selected. It is located in the central section of the proteins.

Consensus pattern[LIVM]-x-G-x(2)-E-G-x-[FY]-x-[FW]-[LIVA]-[TAG]-x-N-[HY]

[1] Handa M., Guidotti G. Biochem. Biophys. Res. Commun. 218:916-923(1996).

[2] Vasconcelos E.G., Ferreira S.T., de Carvalho T.M.U., de Souza W., Kettlun A.M., Mancilla M., Valenzuela M.A., Verjovski-Almeida S. J. Biol. Chem. 271:22139-22145(1996).

771. GTP cyclohydrolase I signatures

PROSITE cross-reference(s); GTP_CYCLOHYDROL_1_1, GTP_CYCLOHYDROL_1_2

GTP cyclohydrolase I (EC 3.5.4.16) catalyzes the biosynthesis of formic acid and dihydroneopterin triphosphate from GTP. This reaction is the first step in the biosynthesis of tetrahydrofolate in prokaryotes, of tetrahydrobiopterin in vertebrates, and of pteridine-containing pigments in insects.

GTP cyclohydrolase I is a protein of from 190 to 250 amino acid residues. The comparison of the sequence of the enzyme from bacterial and eukaryotic sources shows that the structure of this enzyme has been extremely well conserved throughout evolution [1].

Two conserved regions were selected as signature patterns. The first contains a perfectly conserved tetrapeptide which is part of the GTP-binding pocket [2], the second region also contains conserved residues involved in GTP-binding.

Consensus pattern[DEN]-[LIVM](2)-x(2)-[KRNQ]-[DEN]-[LIVM]-x(3)-[ST]-x-C-E- H-H

Consensus pattern[SA]-x-[RK]-x-Q-[LIVM]-Q-E-[RN]-[LI]-[TSN]

[1] Maier J., Witter K., Guetlich M., Ziegler I., Werner T., Ninnemann H. Biochem. Biophys. Res. Commun. 212:705-711(1995).

[2] Nar H., Huber R., Meining W., Schmid C., Weinkauf S., Bacher A. Structure 3:459-466(1995).

772. IlvC. Acetohydroxy acid isomeroreductase

Acetohydroxy acid isomeroreductase catalyses the conversion of acetohydroxy acids into dihydroxy valerates. This reaction is the second in the synthetic pathway of the essential branched side chain amino acids valine and isoleucine. Number of members: 29

[1] Medline: 97361822. The crystal structure of plant acetohydroxy acid isomeroreductase complexed with NADPH, two magnesium ions and a herbicidal transition state analog determined at 1.65 A resolution. Biou V, Dumas R, Cohen-Addad C, Douce R, Job D, Pebay-Peyroula E; EMBO J 1997;16:3405-3415.

773. Prokaryotic membrane lipoprotein lipid attachment site
PROSITE cross-reference(s); PROKAR_LIPOPROTEIN
In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which a glyceride-fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]):

- Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp).
- Escherichia coli lipoprotein-28 (gene nlpA).
- Escherichia coli lipoprotein-34 (gene nlpB).
- Escherichia coli lipoprotein nlpC.
- Escherichia coli lipoprotein nlpD.
- Escherichia coli osmotically inducible lipoprotein B (gene osmB).
- Escherichia coli osmotically inducible lipoprotein E (gene osmE).
- Escherichia coli peptidoglycan-associated lipoprotein (gene pal).
- Escherichia coli rare lipoproteins A and B (genes rplA and rplB).
- Escherichia coli copper homeostasis protein cutF (or nlpE).
- Escherichia coli plasmids traT proteins.
- Escherichia coli Col plasmids lysis proteins.
- A number of Bacillus beta-lactamases.
- Bacillus subtilis periplasmic oligopeptide-binding protein (gene oppA).
- Borrelia burgdorferi outer surface proteins A and B (genes ospA and ospB).
- Borrelia hermsii variable major protein 21 (gene vmp21) and 7 (gene vmp7).
- Chlamydia trachomatis outer membrane protein 3 (gene omp3).

- Fibrobacter succinogenes endoglucanase cel-3.
- Haemophilus influenzae proteins Pal and Pcp.
- Klebsiella pullulunase (gene pulA).
- Klebsiella pullulunase secretion protein pulS.
- Mycoplasma hyorhinis protein p37.
- Mycoplasma hyorhinis variant surface antigens A, B, and C (genes vlpABC).
- Neisseria outer membrane protein H.8.
- Pseudomonas aeruginosa lipopeptide (gene lppL).
- Pseudomonas solanacearum endoglucanase egl.
- Rhodopseudomonas viridis reaction center cytochrome subunit (gene cytC).
- Rickettsia 17 Kd antigen.
- Shigella flexneri invasion plasmid proteins mxiJ and mxiM.
- Streptococcus pneumoniae oligopeptide transport protein A (gene amiA).
- Treponema pallidium 34 Kd antigen.
- Treponema pallidium membrane protein A (gene tmpA).
- Vibrio harveyi chitobiase (gene chb).
- Yersinia virulence plasmid protein yscJ.

- Halocyanin from Natrobacterium pharaonis [4], a membrane associated copper- binding protein. This is the first archaebacterial protein known to be modified in such a fashion).

From the precursor sequences of all these proteins, we derived a consensus pattern and a set of rules to identify this type of post-translational modification.

Consensus pattern{DERK}(6)-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence.

[1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).

[2]Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).

[3]von Heijne G. Protein Eng. 2:531-534(1989).

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[4]Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

774. Aminoacyl-transfer RNA synthetases class-II signatures

PROSITE cross-reference(s); AA_TRNA_LIGASE_II_1; AA_TRNA_LIGASE_II_2

Aminoacyl-tRNA synthetases (EC 6.1.1.-) [1] are a group of enzymes which activate amino acids and transfer them to specific tRNA molecules as the first step in protein biosynthesis. In prokaryotic organisms there are at least twenty different types of aminoacyl-tRNA synthetases, one for each different amino acid. In eukaryotes there are generally two aminoacyl-tRNA synthetases for each different amino acid: one cytosolic form and a mitochondrial form. While all these enzymes have a common function, they are widely diverse in terms of subunit size and of quaternary structure.

The synthetases specific for alanine, asparagine, aspartic acid, glycine, histidine, lysine, phenylalanine, proline, serine, and threonine are referred to as class-II synthetases [2 to 6] and probably have a common folding pattern in their catalytic domain for the binding of ATP and amino acid which is different to the Rossmann fold observed for the class I synthetases [7].

Class-II tRNA synthetases do not share a high degree of similarity, however at least three conserved regions are present [2,5,8]. Signature patterns from two of these regions have been derived.

Consensus pattern[FYH]-R-x-[DE]-x(4,12)-[RH]-x(3)-F-x(3)-[DE]

Consensus pattern[GSTALVF]-{DENQHRKP}-[GSTA]-[LIVMF]-[DE]-R-[LIVMF]-x-[LIVMSTAG]-[LIVMFY]

[1]Schimmel P. Annu. Rev. Biochem. 56:125-158(1987).

[2]Delarue M., Moras D. BioEssays 15:675-687(1993).

[3]Schimmel P. Trends Biochem. Sci. 16:1-3(1991).

[4]Nagel G.M., Doolittle R.F. Proc. Natl. Acad. Sci. U.S.A. 88:8121-8125(1991).

[5]Cusack S., Haertlein M., Leberman R. Nucleic Acids Res. 19:3489-3498(1991).

[6]Cusack S. Biochimie 75:1077-1081(1993).

[7]Cusack S., Berthet-Colominas C., Haertlein M., Nassar N., Leberman R. Nature 347:249-255(1990).

[8]Leveque F., Plateau P., Dessen P., Blanquet S. Nucleic Acids Res. 18:305-312(1990).

775. X. Trans-activation protein X

This protein is found in hepadnaviruses where it is indispensable for replication. Number of members: 91

776. Thymidylate synthase active site

Thymidylate synthase (EC 2.1.1.45) [1,2] catalyzes the reductive methylation of dUMP to dTMP with concomitant conversion of 5,10-methylenetetrahydrofolate to dihydrofolate. Thymidylate synthase plays an essential role in DNA synthesis and is an important target for certain chemotherapeutic drugs.

Thymidylate synthase is an enzyme of about 30 to 35 Kd in most species except in protozoan and plants where it exists as a bifunctional enzyme that includes a dihydrofolate reductase domain.

A cysteine residue is involved in the catalytic mechanism (it covalently binds the 5,6-dihydro-dUMP intermediate). The sequence around the active site of this enzyme is conserved from phages to vertebrates.

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Consensus patternR-x(2)-[LIVM]-x(3)-[FW]-[QN]-x(8,9)-[LV]-x-P-C-[HAVM]-x(3)-[QMT]-[FYW]-x-[LV] [C is the active site residue]

[1] Benkovic S.J. Annu. Rev. Biochem. 49:227-251(1980).

[2] Ross P., O'Gara F., Condon S. Appl. Environ. Microbiol. 56:2156-2163(1990).

777. Glycosyl hydrolases family 31 signatures

It has been shown [1,2,3,E1] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

- Lysosomal alpha-glucosidase (EC 3.2.1.20) (acid maltase) is a vertebrate glycosidase active at low pH, which hydrolyzes alpha(1->4) and alpha(1->6) linkages in glycogen, maltose, and isomaltose.
- Alpha-glucosidase (EC 3.2.1.20) from the yeast Candida tsukunbaensis.

- Alpha-glucosidase (EC 3.2.1.20) (gene malA) from the archebacteria Sulfolobus solfataricus.
- Intestinal sucrase-isomaltase (EC 3.2.1.48 / EC 3.2.1.10) is a vertebrate membrane-bound, multifunctional enzyme complex which hydrolyzes sucrose, maltose and isomaltose. The sucrase and isomaltase domains of the enzyme are homologous (41% of amino acid identity) and have most probably evolved by duplication.
- Glucoamylase 1 (EC 3.2.1.3) (glucan 1,4-alpha-glucosidase) from various fungal species.
- Yeast hypothetical protein YBR229c.
- Fission yeast hypothetical protein SpAC30D11.01c.

An aspartic acid has been implicated [4] in the catalytic activity of sucrase, isomaltase, and lysosomal alpha-glucosidase. The region around this active residue is highly conserved and can be used as a signature pattern. A second region, which contains two conserved cysteines, has been used as an additional signature pattern.

Consensus pattern [GF]-[LIVMF]-W-x-D-M-[NSA]-E [D is the active site residue]
Consensus pattern G-[AV]-D-[LIVMTA]-C-G-[FY]-x(3)-[ST]-x(3)-L-C-x-R-W-x(2)-[LV]-[GSA]-[SA]-F-x-P-F-x-R-[DN]

[1] Henrissat B. Biochem. J. 280:309-316(1991).
[2] Kinsella B.T., Hogan S., Larkin A., Cantwell B.A. Eur. J. Biochem. 202:657-664(1991).
[3] Naim H.Y., Niermann T., Kleinhans U., Hollenberg C.P., Strasser A.W.M. FEBS Lett. 294:109-112(1991).
[4] Hermans M.M.P., Kroos M.A., van Beeumen J., Oostra B.A., Reuser A.J.J. J. Biol. Chem. 266:13507-13512(1991).

778. Urease signatures

Urease (EC 3.5.1.5) is a nickel-binding enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia [1]. Historically, it was the first enzyme to be crystallized (in 1926). It is mainly found in plant seeds, microorganisms and invertebrates. In plants, urease is a hexamer of identical chains. In bacteria [2], it consists of either two or three different subunits (alpha, beta and gamma).

Urease binds two nickel ions per subunit; four histidine, an aspartate and a carbamated-lysine serve as ligands to these metals; an additional histidine is involved in the catalytic mechanism [3].

As signatures for this enzyme, a region was selected that contains two histidine that bind one of the nickel ions and the region of the active site histidine.

Consensus pattern T-[AY]-[GA]-[GAT]-[LIVM]-D-x-H-[LIVM]-H-x(3)-P [The two H's bind nickel]

Consensus pattern [LIVM](2)-[CT]-H-[HN]-L-x(3)-[LIVM]-x(2)-D-[LIVM]-x-F-A [H is the active site residue]

[1] Takishima K., Suga T., Mamiya G. Eur. J. Biochem. 175:151-165(1988).
[2] Mobley H.L.T., Husinger R.P. Microbiol. Rev. 53:85-108(1989).
[3] Jabri E., Carr M.B., Hausinger R.P., Karplus P.A. Science 268:998-1004(1995).

09555296.061900

779. Tyrosine specific protein phosphatases signature and profiles

Tyrosine specific protein phosphatases (EC 3.1.3.48) (PTPase) [1 to 5] are enzymes that catalyze the removal of a phosphate group attached to a tyrosine residue. These enzymes are very important in the control of cell growth, proliferation, differentiation and transformation. Multiple forms of PTPase have been characterized and can be classified into two categories: soluble PTPases and transmembrane receptor proteins that contain PTPase domain(s). The currently known PTPases are listed below:

Soluble PTPases.

- PTPN1 (PTP-1B).
- PTPN2 (T-cell PTPase; TC-PTP).
- PTPN3 (H1) and PTPN4 (MEG), enzymes that contain an N-terminal band 4.1- like domain (see <PDOC00566>) and could act at junctions between the membrane and cytoskeleton.
- PTPN5 (STEP).
- PTPN6 (PTP-1C; HCP; SHP) and PTPN11 (PTP-2C; SH-PTP3; Syp), enzymes which contain two copies of the SH2 domain at its N-terminal extremity. The Drosophila protein corkscrew (gene csw) also belongs to this subgroup.

- PTPN7 (LC-PTP; Hematopoietic protein-tyrosine phosphatase; HePTP).
- PTPN8 (70Z-PEP).
- PTPN9 (MEG2).
- PTPN12 (PTP-G1; PTP-P19).
- Yeast PTP1.
- Yeast PTP2 which may be involved in the ubiquitin-mediated protein degradation pathway.
- Fission yeast pyp1 and pyp2 which play a role in inhibiting the onset of mitosis.
- Fission yeast pyp3 which contributes to the dephosphorylation of cdc2.
- Yeast CDC14 which may be involved in chromosome segregation.
- Yersinia virulence plasmid PTPAses (gene yopH).
- Autographa californica nuclear polyhedrosis virus 19 Kd PTPase.

09595305.050702

Dual specificity PTPases.

- DUSP1 (PTPN10; MAP kinase phosphatase-1; MKP-1); which dephosphorylates MAP kinase on both Thr-183 and Tyr-185.
- DUSP2 (PAC-1), a nuclear enzyme that dephosphorylates MAP kinases ERK1 and ERK2 on both Thr and Tyr residues.
- DUSP3 (VHR).
- DUSP4 (HVH2).
- DUSP5 (HVH3).
- DUSP6 (Pyst1; MKP-3).
- DUSP7 (Pyst2; MKP-X).
- Yeast MSG5, a PTPase that dephosphorylates MAP kinase FUS3.
- Yeast YVH1.
- Vaccinia virus H1 PTPase; a dual specificity phosphatase.

Receptor PTPases.

Structurally, all known receptor PTPases, are made up of a variable length extracellular domain, followed by a transmembrane region and a C-terminal catalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectin type III (FN-III) repeats, immunoglobulin-like domains, MAM domains or carbonic anhydrase-like domains in their extracellular region. The cytoplasmic region generally contains two copies of the

PTPAse domain. The first seems to have enzymatic activity, while the second is inactive but seems to affect substrate specificity of the first. In these domains, the catalytic cysteine is generally conserved but some other, presumably important, residues are not.

In the following table, the domain structure of known receptor PTPases is shown:

	Extracellular				Intracellular
	Ig	FN-3	CAH	MAM	PTPase
Leukocyte common antigen (LCA) (CD45)	0	2	0	0	2
Leukocyte antigen related (LAR)	3	8	0	0	2
Drosophila DLAR	3	9	0	0	2
Drosophila DPTP	2	2	0	0	2
PTP-alpha (LRP)	0	0	0	0	2
PTP-beta	0	16	0	0	1
PTP-gamma	0	1	1	0	2
PTP-delta	0	>7	0	0	2
PTP-epsilon	0	0	0	0	2
PTP-kappa	1	4	0	1	2
PTP-mu	1	4	0	1	2
PTP-zeta	0	1	1	0	2

PTPase domains consist of about 300 amino acids. There are two conserved cysteines, the second one has been shown to be absolutely required for activity. Furthermore, a number of conserved residues in its immediate vicinity have also been shown to be important.

A signature pattern was derived for PTPase domains centered on the active site cysteine.

There are three profiles for PTPases, the first one spans the complete domain and is not specific to any subtype. The second profile is specific to dual-specificity PTPases and the third one to the PTP subfamily.

Consensus pattern [LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY] [C is the active site residue]

Notethe M-phase inducer phosphatases (cdc25-type phosphatase) are tyrosine- protein phosphatases that are not structurally related to the above PTPases.

Notethis documentation entry is linked to both a signature pattern and to profiles. As profiles are much more sensitive than the pattern, you should use them if you have access to the necessary software tools to do so.

[1] Fischer E.H., Charbonneau H., Tonks N.K. Science 253:401-406(1991).
[2] Charbonneau H., Tonks N.K. Annu. Rev. Cell Biol. 8:463-493(1992).
[3] Trowbridge I.S. J. Biol. Chem. 266:23517-23520(1991).
[4] Tonks N.K., Charbonneau H. Trends Biochem. Sci. 14:497-500(1989).
[5] Hunter T. Cell 58:1013-1016(1989).

780. Connexins signatures

Gap junctions [1] are specialized regions of the plasma membrane which consist of closely packed pairs of transmembrane channels, the connexons, through which small molecules diffuse from a cell to a neighboring cell. Each connexon is composed of an hexamer of an integral membrane protein which is often referred to as connexin. In a given species there are a number of different, yet structurally related, tissue specific, forms of connexins. The types of connexins which are currently known are listed below.

- Connexin 56 (Cx56).
- Connexin 50 (Cx50) (lens fiber protein MP70).
- Connexin 46 (Cx46) (alpha-3).
- Connexin 45 (Cx45) (alpha-6).
- Connexin 43 (Cx43) (alpha-1).
- Connexin 40 (Cx40) (alpha-5).
- Connexin 38 (Cx38) (alpha-2).
- Connexin 37 (Cx37) (alpha-4).
- Connexin 33 (Cx33) (alpha-7).
- Connexin 32 (Cx32) (beta-1).
- Connexin 31.1 (Cx31.1) (beta-4).
- Connexin 31 (Cx31) (beta-3).
- Connexin 30.3 (Cx30.3) (beta-5).
- Connexin 26 (Cx26) (beta-2).

Structurally the connexins consist of a short cytoplasmic N-terminal domain, followed by four transmembrane segments that delimit two extracellular and one cytoplasmic loops; the C-terminal domain is cytoplasmic and its length is variable (from 20 residues in Cx26 to 260 residues in Cx56). The schematic representation of this structure is shown below.

```
NH2-***      ***       *************-COOH
    **    **  **    **
    **    **    **   **  Cytoplasmic
  ---**----**-----**----**----------------
    **    **    **   **  Membrane
    **    **    **   **
  ---**----**-----**----**----------------
    **    **    **   **  Extracellular
     ** **      ** **
      **          **
```

The sequences of the two extracellular loops are well conserved. In both loops there are three conserved cysteines which are involved in disulfide bonds. A signature patterns from each of these two loop regions has been built.

Consensus patternC-[DN]-T-x-Q-P-G-C-x(2)-V-C-[FY]-D [The three C's are involved in disulfide bonds] Consensus patternC-x(3,4)-P-C-x(3)-[LIVM]-[DEN]-C-[FY]-[LIVM]-[SA]-[KR]-P [The three C's are involved in disulfide bonds]

[1] Goodenough D.A., Goliger J.A., Paul D.L. Annu. Rev. Biochem. 65:475-502(1996).

781. Gram-positive cocci surface proteins 'anchoring' hexapeptide

Surface proteins from Gram-positive cocci contains a conserved hexapeptide located a few residues downstream of a hydrophobic C-terminal membrane anchor region which is followed by a cluster of basic amino acids [1]. This structure is represented in the following schematic representation:

```
+----------------------------------------+-+--------+-+
| Variable length extracellular domain   |H| Anchor |B|
                                             639
+-----------------------------------------+-+--------+-+
```

'H': conserved hexapeptide.

'B': cluster of basic residues.

It has been proposed that this hexapeptide sequence is responsible for a post-translational modification necessary for the proper anchoring of the proteins which bear it, to the cell wall. Proteins known to contain such hexapeptide are listed below:

- Aggregation substance from streptococcus faecalis (asa1).
- C5a peptidase from Streptococcus pyogenes (scpA).
- C protein alpha-antigen from Streptococcus agalactiae (bca).
- Cell surface antigen I/II (PAC) from Streptococcus mutans.
- Dextranase from Streptococcus downei (dex).
- Fibronectin-binding protein from Staphylococcus aureus (fnbA).
- Fimbrial subunits from Actinomyces naeslundii and viscosus.
- IgA binding protein from Streptococcus pyogenes (arp4).
- IgA binding protein (B antigen) from Streptococcus agalactiae (bag).
- IgG binding proteins from Streptococci and Staphylococcus aureus.
- Internalin A from Listeria monocytogenes (inlA).
- M proteins from streptococci.
- Muramidase-released protein from Streptococcus suis (mrp).
- Nisin leader peptide processing protease from Lactococcus lactis (nisP).
- Protein A from Staphylococcus aureus.
- Trypsin-resistant surface T protein from streptococci.
- Wall-associated protein from Streptococcus mutans (wapA).
- Wall-associated serine proteinases from Lactococcus lactis.

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Consensus patternL-P-x-T-G-[STGAVDE]

[1] Schneewind O., Jones K.F., Fischetti V.A. J. Bacteriol. 172:3310-3317(1990).

782. Gamma-glutamyltranspeptidase signature

Gamma-glutamyltranspeptidase (EC 2.3.2.2) (GGT) [1] catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione. In prokaryotes and eukaryotes, it is an enzyme that consists of two polypeptide chains, a heavy and a light subunit, processed from a single chain precursor. The active site of GGT is known to be located in the light subunit.

The sequences of mammalian and bacterial GGT show a number of regions of high similarity [2]. Pseudomonas cephalosporin acylases (EC 3.5.1.-) that convert 7-beta-(4-carboxybutanamido)-cephalosporanic acid (GL-7ACA) into 7-aminocephalosporanic acid (7ACA) and glutaric acid are evolutionary related to GGT and also show some GGT activity [3]. Like GGT, these GL-7ACA acylases, are also composed of two subunits.

One of the conserved regions correspond to the N-terminal extremity of the mature light chains of these enzymes. This region has been used as a signature pattern.

Consensus patternT-[STA]-H-x-[ST]-[LIVMA]-x(4)-G-[SN]-x-V-[STA]-x-T-x-T-[LIVM]-[NE]-x(1,2)-[FY]-G

09585956.051601

[1] Tate S.S., Meister A. Meth. Enzymol. 113:400-419(1985).

[2] Suzuki H., Kumagai H., Echigo T., Tochikura T. J. Bacteriol. 171:5169-5172(1989).

[3] Ishiye M., Niwa M. Biochim. Biophys. Acta 1132:233-239(1992).

783. Ferrochelatase signature

Ferrochelatase (EC 4.99.1.1) (protoheme ferro-lyase) [1,2] catalyzes the last step in heme biosynthesis: the chelation of a ferrous ion to proto-porphyrin IX, to form protoheme.

In eukaryotes, ferrochelatase is a mitochondrial protein bound to the inner membrane, whose active site faces the mitochondrial matrix. The mature form of eukaryotic ferrochelatase is composed of about 360 amino acids. In bacteria, ferrochelatase (gene hemH) [3] is a protein of from 310 to 380 amino acids.

The human autosomal dominant disease protoporphyria is due to the reduced activity of ferrochelatase.

The signature pattern for this enzyme is based on a conserved region which contains a histidine residue which could be involved in binding iron.

Consensus pattern[LIVMF](2)-x-[ST]-x-H-[GS]-[LIVM]-P-x(4,5)-[DENQKR]-x-G-[DP]-x(1,2)-Y

[1] Labbe-Bois R. J. Biol. Chem. 265:7278-7283(1990).

[2] Brenner D.A., Frasier F. Proc. Natl. Acad. Sci. U.S.A. 88:849-853(1991).

[3] Miyamoto K., Nakahigashi K., Nishimura K., Inokuchi H. J. Mol. Biol. 219:393-398(1991).

784. Cellulose-binding domain, bacterial type

The microbial degradation of cellulose and xylans requires several types of enzyme such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1].

Structurally, cellulases and xylanases generally consist of a catalytic domain joined to a cellulose-binding domain (CBD) by a short linker sequence rich in proline and/or hydroxy-amino acids.

The CBD of a number of bacterial cellulases has been shown to consist of about 105 amino acid residues [2]. Enzymes known to contain such a domain are:

- Endoglucanase (gene end1) from Butyrivibrio fibrisolvens.
- Endoglucanases A (gene cenA) and B (cenB) from Cellulomonas fimi.
- Exoglucanases A (gene cbhA) and B (cbhB) from Cellulomonas fimi.
- Endoglucanase E-2 (gene celB) from Thermomonospora fusca.
- Endoglucanase A (gene celA) from Microbispora bispora.
- Endoglucanases A (gene celA), B (celB) and C (celC) from Pseudomonas fluorescens.
- Endoglucanase A (gene celA) from Streptomyces lividans.
- Exocellobiohydrolase (gene cex) from Cellulomonas fimi.
- Xylanases A (gene xynA) and B (xynB) from Pseudomonas fluorescens.
- Arabinofuranosidase C (EC 3.2.1.55) (xylanase C) (gene xynC) from Pseudomonas fluorescens.
- Chitinase 63 (EC 3.2.1.14) from Streptomyces plicatus.
- Chitinase C from Streptomyces lividans.

The CBD domain is found either at the N-terminal or at the C-terminal extremity of these enzymes. As it is shown in the following schematic representation, there are two conserved cysteines in this CBD domain - one at each extremity of the domain - which have been shown

[3] to be involved in a disulfide bond. There are also four conserved tryptophan residues which could be involved in the interaction of the CBD with polysaccharides.

```
 +------------------------------------------------+
 |                                   |
xCxxxxWxxxxxNxxxWxxxxxxxWxxxxxxxxWNxxxxxGxxxxxxxxxxCx
                        ********
```

'C': conserved cysteine involved in a disulfide bond. '*': position of the pattern.

Consensus patternW-N-[STAGR]-[STDN]-[LIVM]-x(2)-[GST]-x-[GST]-x(2)- [LIVMFT]-[GA]

[1] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).

[2] Meinke A., Gilkes N.R., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Protein Seq. Data Anal. 4:349-353(1991).

[3] Gilkes N.R., Claeyssens M., Aebersold R., Henrissat B., Meinke A., Morrison H.D., Kilburn D.G., Warren R.A.J., Miller R.C. Jr. Eur. J. Biochem. 202:367-377(1991).

785. Amidases signature

It has been shown [1,2,3] that several enzymes from various prokaryotic and eukaryotic organisms which are involved in the hydrolysis of amides (amidases) are evolutionary related. These enzymes are listed below.

- Indoleacetamide hydrolase (EC 3.5.1.-), a bacterial plasmid-encoded enzyme that catalyzes the hydrolysis of indole-3-acetamide (IAM) into indole-3-acetate (IAA), the second step in the biosynthesis of auxins from tryptophan.
- Acetamidase from Emericella nidulans (gene amdS), an enzyme which allows acetamide to be used as a sole carbon or nitrogen source.
- Amidase (EC 3.5.1.4) from Rhodococcus sp. N-774 and Brevibacterium sp. R312 (gene amdA). This enzyme hydrolyzes propionamides efficiently, and also at a lower efficiency, acetamide, acrylamide and indoleacetamide.
- Amidase (EC 3.5.1.4) from Pseudomonas chlororaphis.

- 6-aminohexanoate-cyclic-dimer hydrolase (EC 3.5.2.12) (nylon oligomers degrading enzyme E1) (gene nylA), a bacterial plasmid encoded enzyme which catalyzes the first step in the degradation of 6-aminohexanoic acid cyclic dimer, a by-product of nylon manufacture [4].
- Glutamyl-tRNA(Gln) amidotransferase subunit A [5].
- Mammalian fatty acid amide hydrolase (gene FAAH) [6].
- A putative amidase from yeast (gene AMD2).
- Mycobacterium tuberculosis putative amidases amiA2, amiB2, amiC and amiD.

All these enzymes contain in their central section a highly conserved region rich in glycine, serine, and alanine residues. This region has been used as a signature pattern.

Consensus pattern: G-[GA]-S-[GS]-[GS]-G-x-[GSA]-[GSAVY]-x-[LIVM]-[GSA]-x(6)-[GSAT]-x-[GA]-x-[DE]-x-[GA]-x-S-[LIVM]-R-x-P-[GSAC]

[1] Mayaux J.-F., Cerbelaud E., Soubrier F., Faucher D., Petre D. J. Bacteriol. 172:6764-6773(1990).

[2] Hashimoto Y., Nishiyama M., Ikehata O., Horinouchi S., Beppu T. Biochim. Biophys. Acta 1088:225-233(1991).

[3] Chang T.-H., Abelson J. Nucleic Acids Res. 18:7180-7180(1990).

[4] Tsuchiya K., Fukuyama S., Kanzaki N., Kanagawa K., Negoro S., Okada H. J. Bacteriol. 171:3187-3191(1989).

[5] Curnow A.W., Hong K.W., Yuan R., Kim S.I., Martins O., Winkler W., Henkin T.M., Soll D. Proc. Natl. Acad. Sci. U.S.A. 94:11819-11826(1997).

[6] Cravatt B.F., Giang D.K., Mayfield S.P., Boger D.L., Lerner R.A., Gilula N.B. Nature 384:83-87(1996).

786. Glycosyl hydrolases family 10 active site

The microbial degradation of cellulose and xylans requires several types of enzymes such as endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) [1,2]. Fungi and bacteria produces a spectrum of cellulolytic enzymes (cellulases) and xylanases which, on the basis of sequence similarities, can be classified into families. One of these families is known as the cellulase family F [3] or as the glycosyl hydrolases family 10 [4,E1]. The enzymes which are currently known to belong to this family are listed below.

- Aspergillus awamori xylanase A (xynA).
- Bacillus sp. strain 125 xylanase (xynA).
- Bacillus stearothermophilus xylanase.
- Butyrivibrio fibrisolvens xylanases A (xynA) and B (xynB).
- Caldocellum saccharolyticum bifunctional endoglucanase/exoglucanase (celB). This protein consists of two domains; it is the N-terminal domain, which has exoglucanase activity, which belongs to this family.
- Caldocellum saccharolyticum xylanase A (xynA).
- Caldocellum saccharolyticum ORF4. This hypothetical protein is encoded in the xynABC operon and is probably a xylanase.
- Cellulomonas fimi exoglucanase/xylanase (cex).
- Clostridium stercorarium thermostable celloxylanase.
- Clostridium thermocellum xylanases Y (xynY) and Z (xynZ).
- Cryptococcus albidus xylanase.
- Penicillium chrysogenum xylanase (gene xylP).
- Pseudomonas fluorescens xylanases A (xynA) and B (xynB).
- Ruminococcus flavefaciens bifunctional xylanase XYLA (xynA). This protein consists of three domains: a N-terminal xylanase catalytic domain that belongs to family 11 of glycosyl hydrolases; a central domain composed of short repeats of Gln, Asn an Trp, and a C-terminal xylanase catalytic domain that belongs to family 10 of glycosyl hydrolases.
- Streptomyces lividans xylanase A (xlnA).
- Thermoanaerobacter saccharolyticum endoxylanase A (xynA).
- Thermoascus aurantiacus xylanase.
- Thermophilic bacterium Rt8.B4 xylanase (xynA).

One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [5], in the exoglucanase from Cellulomonas fimi, to be directly involved in glycosidic bond cleavage by acting as a nucleophile. This region has been used as a signature pattern.

Consensus pattern[GTA]-x(2)-[LIVN]-x-[IVMF]-[ST]-E-[LIY]-[DN]-[LIVMF] [E is the active site residue]

[1] Beguin P. Annu. Rev. Microbiol. 44:219-248(1990).
[2] Gilkes N.R., Henrissat B., Kilburn D.G., Miller R.C. Jr., Warren R.A.J. Microbiol. Rev. 55:303-315(1991).
[3] Henrissat B., Claeyssens M., Tomme P., Lemesle L., Mornon J.-P. Gene 81:83-95(1989).
[4] Henrissat B. Biochem. J. 280:309-316(1991).
[5] Tull D., Withers S.G., Gilkes N.R., Kilburn D.G., Warren R.A.J., Aebersold R. J. Biol. Chem. 266:15621-15625(1991).

0959574.091502

787. Fructose-bisphosphate aldolase class-II signatures

Fructose-bisphosphate aldolase (EC 4.1.2.13) [1,2] is a glycolytic enzyme that catalyzes the reversible aldol cleavage or condensation of fructose-1,6- bisphosphate into dihydroxyacetone-phosphate and glyceraldehyde 3-phosphate. There are two classes of fructose-bisphosphate aldolases with different catalytic mechanisms. Class-II aldolases [2], mainly found in prokaryotes and fungi, are homodimeric enzymes which require a divalent metal ion – generally zinc - for their activity.

This family also includes the following proteins:
- Escherichia coli galactitol operon protein gatY which catalyzes the transformation of tagatose 1,6-bisphosphate into glycerone phosphate and D- glyceraldehyde 3-phosphate.
- Escherichia coli N-acetyl galactosamine operon protein agaY which catalyzes the same reaction as that of gatY.

As signature patterns for this class of enzyme, two conserved regions were selected. The first pattern is located in the first half of the sequence and contains two histidine residues that have been shown [4] to be involved in binding a zinc ion. The second is located in the C-terminal section and contains clustered acidic residues and glycines.

Consensus pattern[FYVMT]-x(1,3)-[LIVMH]-[APN]-[LIVM]-x(1,2)-[LIVM]-H-x-D-H-[GACH] [The two H's are zinc ligands]
Consensus pattern[LIVM]-E-x-E-[LIVM]-G-x(2)-[GM]-[GSTA]-x-E

[1] Perham R.N. Biochem. Soc. Trans. 18:185-187(1990).

[2] Marsh J.J., Lebherz H.G. Trends Biochem. Sci. 17:110-113(1992).

[3] von der Osten C.H., Barbas C.F. III, Wong C.-H., Sinskey A.J. Mol. Microbiol. 3:1625-1637(1989).

[4] Berry A., Marshall K.E. FEBS Lett. 318:11-16(1993).

788. Prolyl oligopeptidase family serine active site

The prolyl oligopeptidase family [1,2,3] consist of a number of evolutionary related peptidases whose catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. The known members of this family are listed below.

- Prolyl endopeptidase (EC 3.4.21.26) (PE) (also called post-proline cleaving enzyme). PE is an enzyme that cleaves peptide bonds on the C-terminal side of prolyl residues. The sequence of PE has been obtained from a mammalian species (pig) and from bacteria (Flavobacterium meningosepticum and Aeromonas hydrophila); there is a high degree of sequence conservation between these sequences.
- Escherichia coli protease II (EC 3.4.21.83) (oligopeptidase B) (gene prtB) which cleaves peptide bonds on the C-terminal side of lysyl and argininyl residues.
- Dipeptidyl peptidase IV (EC 3.4.14.5) (DPP IV). DPP IV is an enzyme that removes N-terminal dipeptides sequentially from polypeptides having unsubstituted N-termini provided that the penultimate residue is proline.
- Yeast vacuolar dipeptidyl aminopeptidase A (DPAP A) (gene: STE13) which is responsible for the proteolytic maturation of the alpha-factor precursor.
- Yeast vacuolar dipeptidyl aminopeptidase B (DPAP B) (gene: DAP2).
- Acylamino-acid-releasing enzyme (EC 3.4.19.1) (acyl-peptide hydrolase). This enzyme catalyzes the hydrolysis of the amino-terminal peptide bond of an N-acetylated protein to generate a N-acetylated amino acid and a protein with a free amino-terminus.

A conserved serine residue has experimentally been shown (in E.coli protease II as well as in pig and bacterial PE) to be necessary for the catalytic mechanism. This serine, which is part of the catalytic triad (Ser, His, Asp), is generally located about 150 residues away from the C-terminal extremity of these enzymes (which are all proteins that contains about 700 to 800 amino acids).

Consensus patternD-x(3)-A-x(3)-[LIVMFYW]-x(14)-G-x-S-x-G-G-[LIVMFYW](2) [S is the active site residue]

Note these proteins belong to families S9A/S9B/S9C in the classification of peptidases [4,E1].

[1] Rawlings N.D., Polgar L., Barrett A.J. Biochem. J. 279:907-911(1991).
[2] Barrett A.J., Rawlings N.D. Biol. Chem. Hoppe-Seyler 373:353-360(1992).
[3] Polgar L., Szabo E.
Biol. Chem. Hoppe-Seyler 373:361-366(1992).
[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

789. Formate--tetrahydrofolate ligase signatures

Formate--tetrahydrofolate ligase (EC 6.3.4.3) (formyltetrahydrofolate synthetase) (FTHFS) is one of the enzymes participating in the transfer of one-carbon units, an essential element of various biosynthetic pathways. In many of these processes the transfers of one-carbon units are mediated by the coenzyme tetrahydrofolate (THF). Various reactions generate one-carbon derivatives of THF which can be interconverted between different oxidation states by FTHFS, methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5) and methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9).

In eukaryotes the FTHFS activity is expressed by a multifunctional enzyme, C-1-tetrahydrofolate synthase (C1-THF synthase), which also catalyzes the dehydrogenase and cyclohydrolase activities. Two forms of C1-THF synthases are known [1], one is located in the mitochondrial matrix, while the second one is cytoplasmic. In both forms the FTHFS domain consist of about 600 amino acid residues and is located in the C-terminal section of C1-THF synthase. In prokaryotes FTHFS activity is expressed by a monofunctional homotetrameric enzyme of about 560 amino acid residues [2].

The sequence of FTHFS is highly conserved in all forms of the enzyme. As signature patterns, two regions that are almost perfectly conserved were selected. The first one is a glycine-rich segment located in the N-terminal part of FTHFS and which could be part of an ATP-binding domain [2]. The second pattern is located in the central section of FTHFS.

Consensus patternG-[LIVM]-K-G-G-A-A-G-G-G-Y
Consensus patternV-A-T-[IV]-R-A-L-K-x-[HN]-G-G

[1] Shannon K.W., Rabinowitz J.C. J. Biol. Chem. 263:7717-7725(1988).
[2] Lovell C.R., Przybyla A., Ljungdahl L.G. Biochemistry 29:5687-5694(1990).

790. Transthyretin signatures

Transthyretin (prealbumin) [1] is a thyroid hormone-binding protein that seems to transport thyroxine (T4) from the bloodstream to the brain. It is a protein of about 130 amino acids that assembles as a homotetramer and forms an internal channel that binds thyroxine. Transthyretin is mainly synthesized in the brain choroid plexus. In humans, variants of the protein are associated with distinct forms of amyloidosis.

The sequence of transthyretin is highly conserved in vertebrates. A number of uncharacterized proteins also belong to this family:

- Escherichia coli hypothetical protein yedX.
- Bacillus subtilis hypothetical protein yunM.
- Caenorhabditis elegans hypothetical protein R09H10.3.
- Caenorhabditis elegans hypothetical protein ZK697.8.

Two regions were selected as signature patterns. The first located in the N-terminal extremity starts with a lysine known to be involved in binding T4. The second pattern is located in the C-terminal extremity.

Consensus pattern[KH]-[IV]-L-[DN]-x(3)-G-x-P-A-x(2)-[IV]-x-[IV] [The K binds thyroxine]
Consensus patternY-[TH]-[IV]-[AP]-x(2)-L-S-[PQ]-[FYW]-[GS]-[FY]-[QS]

[1] Schreiber G., Richardson S.J. Comp. Biochem. Physiol. 116B:137-160(1997).

791. Dihydropteroate synthase signatures

09550521.051602

All organisms require reduced folate cofactors for the synthesis of a variety of metabolites. Most microorganisms must synthesize folate de novo because they lack the active transport system of higher vertebrate cells which allows these organisms to use dietary folates. Enzymes that are involved in the biosynthesis of folates are therefore the target of a variety of antimicrobial agents such as trimethoprim or sulfonamides.

Dihydropteroate synthase (EC 2.5.1.15) (DHPS) catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropteridine pyrophosphate to para-aminobenzoic acid to form 7,8-dihydropteroate. This is the second step in the three steps pathway leading from 6-hydroxymethyl-7,8-dihydropterin to 7,8-dihydrofolate. DHPS is the target of sulfonamides which are substrates analog that compete with para-aminobenzoic acid.

Bacterial DHPS (gene sul or folP) [1] is a protein of about 275 to 315 amino acid residues which is either chromosomally encoded or found on various antibiotic resistance plasmids. In the lower eukaryote Pneumocystis carinii, DHPS is the C-terminal domain of a multifunctional folate synthesis enzyme (gene fas) [2].

Two signature patterns for DHPS were developed, the first signature is located in the N-terminal section of these enzymes, while the second signature is located in the central section.

Consensus pattern[LIVM]-x-[AG]-[LIVMF](2)-N-x-T-x-D-S-F-x-D-x-[SG]
Consensus pattern[GE]-[SA]-x-[LIVM](2)-D-[LIVM]-G-[GP]-x(2)-[STA]-x-P

[1] Slock J., Stahly D.P., Han C.-Y., Six E.W., Crawford I.P. J. Bacteriol. 172:7211-7226(1990).
[2] Volpes F., Dyer M., Scaife J.G., Darby G., Stammers D.K., Delves C.J. Gene 112:213-218(1992).

792. Phosphatidylinositol 3- and 4-kinases signatures

Phosphatidylinositol 3-kinase (PI3-kinase) (EC 2.7.1.137) [1] is an enzyme that phosphorylates phosphoinositides on the 3-hydroxyl group of the inositol ring. The exact function of the three products of PI3-kinase - PI-3-P, PI-3,4-P(2) and PI-3,4,5-P(3) - is not yet known, although it is proposed that they function as second messengers in cell signalling. Currently, three forms of PI3-kinase are known:

- The mammalian enzyme which is a heterodimer of a 110 Kd catalytic chain (p110) and an 85 Kd subunit (p85) which allows it to bind to activated tyrosine protein kinases. There are at least two different types of p100 subunits (alpha and beta).
- Yeast TOR1/DRR1 and TOR2/DRR2 [2], PI3-kinases required for cell cycle activation. Both are proteins of about 280 Kd.
- Yeast VPS34 [3], a PI3-kinase involved in vacuolar sorting and segregation. VPS34 is a protein of about 100 Kd.
- Arabidopsis thaliana and soybean VPS34 homologs.

Phosphatidylinositol 4-kinase (PI4-kinase) (EC 2.7.1.67) [4] is an enzyme that acts on phosphatidylinositol (PI) in the first committed step in the production of the second messenger inositol-1,4,5,-trisphosphate. Currently the following forms of PI4-kinases are known:

- Human PI4-kinase alpha.
- Yeast PIK1, a nuclear protein of 120 Kd.
- Yeast STT4, a protein of 214 Kd.

The PI3- and PI4-kinases share a well conserved domain at their C-terminal section; this domain seems to be distantly related to the catalytic domain of protein kinases [2]. Two signature patterns were developed from the best conserved parts of this domain.

Four additional proteins belong to this family:

- Mammalian FKBP-rapamycin associated protein (FRAP) [5], which acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex.
- Yeast protein ESR1 [6] which is required for cell growth, DNA repair and meiotic recombination.
- Yeast protein TEL1 which is involved in controlling telomere length.
- Yeast hypothetical protein YHR099w, a distantly related member of this family.
- Fission yeast hypothetical protein SpAC22E12.16C.

Consensus pattern[LIVMFAC]-K-x(1,3)-[DEA]-[DE]-[LIVMC]-R-Q-[DE]-x(4)-Q

Consensus pattern[GS]-x-[AV]-x(3)-[LIVM]-x(2)-[FYH]-[LIVM](2)-x-[LIVMF]-x-D-R-H-x(2)-N

[1] Hiles I.D., Otsu M., Volinia S., Fry M.J., Gout I., Dhand R., Panayotou G., Ruiz-Larrea F., Thompson A., Totty N.F., Hsuan J.J., Courtneidge S.A., Parker P.J., Waterfield M.D. Cell 70:419-429(1992).

[2] Kunz J., Henriquez R., Schneider U., Deuter-Reinhard M., Movva N., Hall M.N. Cell 73:585-596(1993).

[3] Schu P.V., Takegawa K., Fry M.J., Stack J.H., Waterfield M.D., Emr S.D. Science 260:88-91(1993).

[4] Garcia-Bustos J.F., Marini F., Stevenson I., Frei C., Hall M.N. EMBO J. 13:2352-2361(1994).

[5] Brown E.J., Albers M.W., Shin T.B., Ichikawa K., Keith C.T., Lane W.S., Schreiber S.L. Nature 369:756-758(1994).

[6] Kato R., Ogawa H. Nucleic Acids Res. 22:3104-3112(1994).

793. FAD-dependent glycerol-3-phosphate dehydrogenase signatures

FAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) (GPD) catalyzes the conversion of glycerol-3-phosphate into dihydroxyacetone phosphate. In bacteria [1] it is associated with the utilization of glycerol coupled to respiration. In Escherichia coli, two isozymes are known: one expressed under anaerobic conditions (gene glpA) and one in aerobic conditions (gene glpD). In eukaryotes, a mitochondrial form of GPD participates in the glycerol phosphate shuttle in conjunction with an NAD-dependent cytoplasmic GPD (EC 1.1.1.8) [2,3].

These enzymes are proteins of about 60 to 70 Kd which contain a probable FAD-binding domain in their N-terminal extremity. The mammalian enzyme differs from the bacterial or yeast proteins by having an EF-hand calcium-binding region (See <PDOC00018>) in its C-terminal extremity.

Two signature patterns were developed. One based on the first half of the FAD-binding domain and one which corresponds to a conserved region in the central part of these enzymes.

Consensus pattern[IV]-G-G-G-x(2)-G-[STACV]-G-x-A-x-D-x(3)-R-G

Consensus patternG-G-K-x(2)-[GSTE]-Y-R-x(2)-A

[1] Austin D., Larson T.J. J. Bacteriol. 173:101-107(1991).

[2] Roennow B., Kielland-Brandt M.C. Yeast 9:1121-1130(1993).

[3] Brown L.J., McDonald M.J., Lehn D.A., Moran S.M. J. Biol. Chem. 269:14363-14366(1994).

794. NOL1/NOP2/sun family signature

The following proteins seems to be evolutionary related:

- Mammalian proliferating-cell nucleolar antigen p120 (gene NOL1) which may play a role in the regulation of the cell cycle and the increased nucleolar activity that is associated with the cell proliferation.
- Yeast nucleolar protein NOP2 (or YNA1) which could be involved in nucleolar function during the onset of growth, and in the maintenance of nucleolar structure.
- Yeast hypothetical protein YBL024w.
- Bacterial protein sun (also known as fmu).
- Escherichia coli hypothetical protein yebU.
- Mycobacterium tuberculosis hypothetical protein MtCY21B4.24.
- Methanococcus jannaschii hypothetical protein MJ0026.

NOL1 is a protein of 855 residues, NOP2 consists of 618 residues, YBL024w of 684, sun is a protein of about 430 to 450 residues and MJ026 has 274 residues. They share a conserved central domain which contains some highly conserved regions. One of these regions was selected as a signature pattern.

Consensus pattern[FV]-D-[KRA]-[LIVMA]-L-x-D-[AV]-P-C-[ST]-[GA]

795. moaA / nifB / pqqE family signature

A number of proteins involved in the biosynthesis of metallo cofactors have been shown [1,2] to be evolutionary related. These proteins are:

- Bacterial and archebacterial protein moaA, which is involved in the biosynthesis of the molybdenum cofactor (molybdopterin; MPT).
- Arabidopsis thaliana cnx2, a protein involved in molybdopterin biosynthesis and which is highlys similar to moaA.
- Bacillus subtilis narA, which seems to be the moaA ortholog in that bacteria.

- Bacterial protein nifB (or fixZ) which is involved in the biosynthesis of the nitrogenase iron-molybdenum cofactor.
- Bacterial protein pqqE which is involved in the biosynthesis of the cofactor pyrrolo-quinoline-quinone (PQQ).
- Pyrococcus furiosus cmo, a protein involved in the synthesis of a molybdopterin-based tungsten cofactor.
- Caenorhabditis elegans hypothetical protein F49E2.1.

All these proteins share, in their N-terminal region, a conserved domain that contains three cysteines. In moaA, these cysteines have been shown [1] to be important for the biological activity. They could be inolved in the binding of an iron-sulfur cluster.

Consensus pattern[LIV]-x(3)-C-[NP]-[LIVMF]-[QRS]-C-x-[FYM]-C [The three C's are putative Fe-S ligands

[1] Menendez C., Igloi G., Henninger H., Brandsch R. Arch. Microbiol. 164:142-151(1995).
[2] Hoff T., Schnorr K.M., Meyer C., Caboche M. J. Biol. Chem. 270:6100-6107(1995).

796. Forkhead-associated (FHA) domain profile

The forkhead-associated (FHA) domain [1,E1] is a putative nuclear signalling domain found in a variety of otherwise unrelated proteins. The FHA domain comprise approximately 55 to 75 amino acids and contains three highly conserved blocks separated by divergent spacer regions. Currently it has been found in the following proteins:

- Four transcription factors that also contain a forkhead (FH) domain: mouse myocyte nuclear factor 1 (MNF1), yeast transcription factor FHL1, which probably controls pre-mRNA processing, and yeast FKH1 and FKH2. In those protein the FHA domain is located N-terminal of the DNA-binding FH domain.
- Kinase-associated protein phosphatase (KAPP) from Arabidopsis thaliana, a protein which specifically interacts with the receptor-type Ser/Thr-kinase RLK5. In KAPP, the FHA domain maps to a region that interacts with the receptor-type protein kinase RLK5 only if the kinase is phosphorylated on serine residues [2].

- Two protein kinases from yeast that are involved in mediating the nuclear response to DNA damage: DUN1 and SPK1/SAD1 [3]. The latter is the only known protein containing two copies of the FHA domain.
- Protein kinase cds1 from fission yeast contains a FHA domain and might be the ortholog of SPK1.
- Protein kinase MEK1 from yeast, which is involved in meiotic recombination.
- Human nuclear antigen Ki67 which is expressed only in proliferating cells.
- Yeast hypothetical protein YHR115c, which contains a RING-finger C-terminal of the FHA domain.
- Yeast hypothetical proteins L8083.1 and 9346.10, which contain an extensive coiled-coil region C-terminal of the FHA domain.
- Caenorhabditis elegans hypothetical protein ZK632.2.
- Caenorhabditis elegans hypothetical protein C01G6.5.
- FraH from the prokaryote Anabaena, which contains a zinc-finger motif N-terminal of the FHA domain.
- An ORF from the bacterium Streptomyces, which is on the opposite strand of the protein kinase pks1, overlapping the ORF of the kinase.

[1] Hofmann K.O., Bucher P. Trends Biochem. Sci. 20:347-349(1995).
[2] Stone J.M., Collinge M.A., Smith R.D., Horn M.A., Walker J.C. Science 266:793-795(1994).
[3] Navas T.A., Zhou Z., Elledge S.J. Cell 80:29-39(1995).

797. Ald_Xan_dh_C

Aldehyde oxidase and xanthine dehydrogenase, C terminus

[1] Romao MJ, Archer M, Moura I, Moura JJ, LeGall J, Engh R, Schneider M, Hof P, Huber R; Medline: 96072968 "Crystal structure of the xanthine oxidase-related aldehyde oxido-reductase from D. gigas." Science 1995;270:1170-1176.

Number of members: 54

798. Glyco_hydro_38

Glycosyl hydrolases family 38

Glycosyl hydrolases are key enzymes of carbohydrate metabolism.

Number of members: 20

[1] Henrissat B; Medline: 98313424; “Glycosidase families” Biochem Soc Trans 1998;26:153-156.

799. HECT

HECT-domain (ubiquitin-transferase).

The name HECT comes from Homologous to the E6-AP Carboxyl Terminus.

Number of members: 43

[1] Huibregtse JM, Scheffner M, Beaudenon S, Howley PM; Medline: 95223981; “A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase.” Proc Natl Acad Sci U S A 1995;92:2563-2567.

800. HRDC

HRDC domain

The HRDC (Helicase and RNase D C-terminal) domain has a putative role in nucleic acid binding. Mutations in the HRDC domain cause human disease.

Number of members: 19

[1] Morozov V, Mushegian AR, Koonin EV, Bork P; Medline: 98060076; “A putative nucleic acid-binding domain in Bloom's and Werner's syndrome helicases” Trends Biochem Sci 1997;22:417-418.

801. Integrase

Integrase mediates integration of a DNA copy of the viral genome into the host chromosome. Integrase is composed of three domains. The amino-terminal domain is a zinc binding domain. The central domain is the catalytic domain [1].The carboxyl terminal domain is a DNA binding domain [2].

Number of members: 581

[1] Dyda F, Hickman AB, Jenkins TM, Engelman A, Craigie R, Davies DR; Medline: 95099322. “Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases.” Science 1994;266:1981-1986.
[2] Lodi PJ, Ernst JA, Kuszewski J, Hickman AB, Engelman A, Craigie R, Clore GM, Gronenborn AM; Medline: 95359147; “Solution structure of the DNA binding domain of HIV-1 integrase.” Biochemistry 1995;34:9826-9833

09555956.061502

802. lig_chan
Ligand-gated ion channel

This family includes the four transmembrane regions of the ionotropic glutamate receptors and NMDA receptors.

Number of members: 128

[1] Tong G, Shepherd D, Jahr CE; Medline: 95184014; “Synaptic desensitization of NMDA receptors by calcineurin.” Science 1995;267:1510-1512.

803. RhoGAP
RhoGAP domain

GTPase activator proteins towards Rho/Rac/Cdc42-like small GTPases.

Number of members: 97

[1] Musacchio A, Cantley LC, Harrison SC; Medline: 97121392; “Crystal structure of the breakpoint cluster region-homology domain from phosphoinositide 3-kinase p85 alpha subunit.” Proc Natl Acad Sci U S A 1996;93:14373-14378.

[2] Barrett T, Xiao B, Dodson EJ, Dodson G, Ludbrook SB, Nurmahomed K, Gamblin SJ, Musacchio A, Smerdon SJ, Eccleston JF; Medline: 97162209; "The structure of the GTPase-activating domain from p50rhoGAP." Nature 1997;385:458-461.

[3] Rittinger K, Walker PA, Eccleston JF, Nurmahomed K, Owen D, Laue E, Gamblin SJ, Smerdon SJ; Medline: 97404320; "Crystal structure of a small G protein in complex with the GTPase-activating protein rhoGAP." Nature 1997;388:693-697.

[4] Boguski MS, McCormick F; Medline: 94081948; "Proteins regulating Ras and its relatives." Nature 1993;366:643-654.

804. vwd

von Willebrand factor type D domain

[1] Bork P; Medline: 93327926; "The modular architecture of a new family of growth regulators related to connective tissue growth factor." FEBS lett 1993;327:125-130.

Number of members: 92

805. zf-C4_Topoisom

Topoisomerase DNA binding C4 zinc finger

[1] Tse-Dinh YC, Beran-Steed RK; Medline: 89034032; "Escherichia coli DNA topoisomerase I is a zinc metalloprotein with three repetitive zinc-binding domains." J Biol Chem 1988;263:15857-15859.

[2] Ahumada A, Tse-Dinh YC; Medline: 99011409; "The Zn(II) binding motifs of E. coli DNA topoisomerase I is part of a high-affinity DNA binding domain." Biochem Biophys Res Commun 1998;251:509-514.

Number of members: 51

806. AIRC

AIR carboxylase

Members of this family catalyse the decarboxylation of 1-(5-phosphoribosyl)-5-amino-4-imidazole-carboxylate (AIR). This family catalyse the sixth step of de novo purine biosynthesis. Some members of this family contain two copies of this domain. Number of members: 35

807. Bromodomain signature and profile

PROSITE cross-reference(s): PS00633; BROMODOMAIN_1, PS50014; BROMODOMAIN_2

The bromodomain [1,2,3] is a conserved region of about 70 amino acids found in the following proteins:

- Higher eukaryotes transcription initiation factor TFIID 250 Kd subunit (TBP-associated factor p250) (gene CCG1). P250 associated with the TFIID TATA-box binding protein and seems essential for progression of the G1 phase of the cell cycle.
- Human RING3, a protein of unknown function encoded in the MHC class II locus.
- Mammalian CREB-binding protein (CBP), which mediates cAMP-gene regulation by binding specifically to phosphorylated CREB protein.
- Drosophila female sterile homeotic protein (gene fsh), required maternally for proper expression of other homeotic genes involved in pattern formation, such as Ubx.
- Drosophila brahma protein (gene brm), a protein required for the activation of multiple homeotic genes.
- Mammalian homologs of brahma. In human, three brahma-like proteins are known: SNF2a(hBRM), SNF2b, and BRG1.
- Human BS69, a protein that binds to adenovirus E1A and inhibits E1A transactivation
- Human peregrin (or Br140).
- Yeast BDF1 [3], a transcription factor involved in the expression of a broad class of genes including snRNAs.
- Yeast GCN5, a general transcriptional activator operating in concert with certain other DNA-binding transcriptional activators, such as GCN4, HAP2/3/4 or ADA2.
- Yeast NPS1/STH1, involved in G(2) phase control in mitosis.
- Yeast SNF2/SWI2, which is part of a complex with the SNF5, SNF6, SWI3 and ADR6/SWI1 proteins. This SWI-complex is involved in transcriptional activation.
- Yeast SPT7, a transcriptional activator of Ty elements and possibly other genes.

09555953.052501

- Caenorhabditis elegans protein cbp-1.
- Yeast hypothetical protein YGR056w.
- Yeast hypothetical protein YKR008w.
- Yeast hypothetical protein L9638.1.

Some proteins contain a region which, while similar to some extent to a classical bromodomain, diverges from it by either lacking part of the domain or because of an insertion. These proteins are:

- Mammalian protein HRX (also known as All-1 or MLL), a protein involved in translocations leading to acute leukemias and which possibly acts as a transcriptional regulatory factor. HRX contains a region similar to the C- terminal half of the bromodomain.
- Caenorhabditis elegans hypothetical protein ZK783.4. The bromodomain of this protein has a 23 amino-acid insertion.
- Yeast protein YTA7. This protein contains a region with significant similarity to the C-terminal half of the bromodomain. As it is a member of the AAA family (see <PDOC00572>) it is also in a functionally different context.

The above proteins generally contain a single bromodomain, but some of them contain two copies, this is the case of BDF1, CCG1, fsh, RING3, YKR008w and L9638.1.

The exact function of this domain is not yet known but it is thought to be involved in protein-protein interactions and it may be important for the assembly or activity of multicomponent complexes involved in transcriptional activation.

The consensus pattern that has been developed spans a major part of the bromodomain; a more sensitive detection is available through the use of a profile which spans the whole domain.

Consensus pattern[STANVF]-x(2)-F-x(4)-[DNS]-x(5,7)-[DENQTF]-Y-[HFY]-x(2)-[LIVMFY]-x(3)-[LIVM]-x(4)-[LIVM]-x(6,8)-Y-x(12,13)-[LIVM]-x(2)-N-[SACF]-x(2)-[FY]

References
[1] Haynes S.R., Doolard C., Winston F., Beck S., Trowsdale J., Dawid I.B. Nucleic Acids Res. 20:2693-2603(1992).
[2] Tamkun J.W., Deuring R., Scott M.P., Kissinger M., Pattatucci A.M., Kaufman T.C., Kennison J.A. Cell 68:561-572(1992).
[3] Tamkun J.W. Curr. Opin. Genet. Dev. 5:473-477(1995).

808. (CH) Actinin-type actin-binding domain signatures
PROSITE cross-reference(s): PS00019; ACTININ_1, PS00020; ACTININ_2

Alpha-actinin is a F-actin cross-linking protein which is thought to anchoractin to a variety of intracellular structures [1]. The actin-binding domain of alpha-actinin seems to reside in the first 250 residues of the protein. A similar actin-binding domain has been found in the N-terminal region of many different actin-binding proteins [2,3]:

- In the beta chain of spectrin (or fodrin).
- In dystrophin, the protein defective in Duchenne muscular dystrophy (DMD) and which may play a role in anchoring the cytoskeleton to the plasma membrane.
- In the slime mold gelation factor (or ABP-120).
- In actin-binding protein ABP-280 (or filamin), a protein that link actin filaments to membrane glycoproteins.
- In fimbrin (or plastin), an actin-bundling protein. Fimbrin differs from the above proteins in that it contains two tandem copies of the actin-binding domain and that these copies are located in the C-terminal part of the protein.

Two conserved regions were selected as signature patterns for this type of main. The first of this region is located at the beginning of the domain, hile the second one is located in the central section and has been shown to be essential for the binding of actin.

Consensus pattern[EQ]-x(2)-[ATV]-[FY]-x(2)-W-x-N
Consensus pattern[LIVM]-x-[SGN]-[LIVM]-[DAGHE]-[SAG]-x-[DNEAG]-[LIVM]-x-[DEAG]-x(4)-[LIVM]-x-[LM]-[SAG]-[LIVM]-[LIVMT]-W-x- [LIVM](2)

09555358.061502

[1] Schleicher M., Andre E., Harmann A., Noegel A.A. Dev. Genet. 9:521-530(1988).

[2] Matsudaira P. Trends Biochem. Sci. 16:87-92(1991).

[3] Dubreuil R.R. BioEssays 13:219-226(1991).

809. (COX1) Heme-copper oxidase subunit I, copper B binding region signature

PROSITE cross-reference(s): PS00077; COX1

Heme-copper respiratory oxidases [1] are oligomeric integral membrane protein complexes that catalyze the terminal step in the respiratory chain: they transfer electrons from cytochrome c or a quinol to oxygen. Some terminal oxidases generate a transmembrane proton gradient across the plasma membrane (prokaryotes) or the mitochondrial inner membrane (eukaryotes). The enzyme complex consists of 3-4 subunits (prokaryotes) up to 13 polypeptides (mammals) of which only the catalytic subunit (equivalent to mammalian subunit 1 (CO I)) is found in all heme-copper respiratory oxidases. The presence of a bimetallic center (formed by a high-spin heme and copper B) as well as a low-spin heme, both ligated to six conserved histidine residues near the outer side of four transmembrane spans within CO I is common to all family members [2-4].

09595659.020802

In contrary to eukaryotes the respiratory chain of prokaryotes is branched to multiple terminal oxidases. The enzyme complexes vary in heme and copper composition, substrate type and substrate affinity. The different respiratory oxidases allow the cells to customize their respiratory systems according a variety of environmental growth conditions [1].

Recently also a component of an anaerobic respiratory chain has been found to contain the copper B binding signature of this family: nitric oxide reductase (NOR) exists in denitrifying species of Archae and Eubacteria.

Enzymes that belong to this family are:

- Mitochondrial-type cytochrome c oxidase (EC 1.9.3.1) which uses cytochrome c as electron donor. The electrons are transferred via copper A (Cu(A)) and heme a to the bimetallic center of CO I that is formed by a pentacoordinated heme a and copper B (Cu(B)). Subunit 1 contains 12 transmembrane regions. Cu(B) is said to be ligated to three of the conserved histidine residues within the transmembrane segments 6 and 7.

- Quinol oxidase from prokaryotes that transfers electrons from a quinol to the binuclear center of polypeptide I. This category of enzymes includes Escherichia coli cytochrome O terminal oxidase complex which is a component of the aerobic respiratory chain that predominates when cells are grown at high aeration.
- FixN, the catalytic subunit of a cytochrome c oxidase expressed in nitrogen-fixing bacteroids living in root nodules. The high affinity for oxygen allows oxidative phosphorylation under low oxygen concentrations. A similar enzyme has been found in other purple bacteria.
- Nitric oxide reductase (EC 1.7.99.7) from Pseudomonas stutzeri. NOR reduces nitrate to dinitrogen. It is a heterodimer of norC and the catalytic subunit norB. The latter contains the 6 invariant histidine residues and 12 transmembrane segments [5].

As a signature pattern the copper-binding region was used.

Consensus pattern[YWG]-[LIVFYWTA](2)-[VGS]-H-[LNP]-x-V-x(44,47)-H-H [The three H's are copper B ligands]

Notecytochrome bd complexes do not belong to this family.

[1]
Garcia-Horsman J.A., Barquera B., Rumbley J., Ma J., Gennis R.B.
J. Bacteriol. 176:5587-5600(1994).
[2]
Castresana J., Luebben M., Saraste M., Higgins D.G.
EMBO J. 13:2516-2525(1994).
[3]
Capaldi R.A., Malatesta F., Darley-Usmar V.M.
Biochim. Biophys. Acta 726:135-148(1983).

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[4]
Holm L., Saraste M., Wikstrom M.
EMBO J. 6:2819-2823(1987).
[5]
Saraste M., Castresana J.
FEBS Lett. 341:1-4(1994).

810. (dehydrog_molyb) Eukaryotic molybdopterin oxidoreductases signature
PROSITE cross-reference(s): PS00559; MOLYBDOPTERIN_EUK

A number of different eukaryotic oxidoreductases that require and bind a molybdopterin cofactor have been shown [1] to share a few regions of sequence similarity. These enzymes are:

- Xanthine dehydrogenase (EC 1.1.1.204), which catalyzes the oxidation of xanthine to uric acid with the concomitant reduction of NAD. Structurally, this enzyme of about 1300 amino acids consists of at least three distinct domains: an N-terminal 2Fe-2S ferredoxin-like iron-sulfur binding domain (see <PDOC00175>), a central FAD/NAD-binding domain and a C-terminal Mo-pterin domain.
- Aldehyde oxidase (EC 1.2.3.1), which catalyzes the oxidation aldehydes into acids. Aldehyde oxidase is highly similar to xanthine dehydrogenase in its sequence and domain structure.
- Nitrate reductase (EC 1.6.6.1), which catalyzes the reduction of nitrate to nitrite. Structurally, this enzyme of about 900 amino acids consists of an N-terminal Mo-pterin domain, a central cytochrome b5-type heme-binding domain (see <PDOC00170>) and a C-terminal FAD/NAD-binding cytochrome reductase domain.
- Sulfite oxidase (EC 1.8.3.1), which catalyzes the oxidation of sulfite to sulfate. Structurally, this enzyme of about 460 amino acids consists of an N-terminal cytochrome b5-binding domain followed by a Mo-pterin domain.

There are a few conserved regions in the sequence of the molybdopterin-binding domain of these enzymes. The pattern uses to detect these proteins is based on one of them. It contains a cysteine residue which could be involved in binding the molybdopterin cofactor.

Consensus pattern[GA]-x(3)-[KRNQHT]-x(11,14)-[LIVMFYWS]-x(8)-[LIVMF]-x-C-x(2)-[DEN]-R-x(2)-[DE]

[1]
Wootton J.C., Nicolson R.E., Cock J.M., Walters D.E., Burke J.F., Doyle W.A., Bray R.C.
Biochim. Biophys. Acta 1057:157-185(1991).

811. (DNA_ligase) ATP-dependent DNA ligase signatures
PROSITE cross-reference(s): PS00697; DNA_LIGASE_A1, PS00333; DNA_LIGASE_A2

DNA ligase (polydeoxyribonucleotide synthase) is the enzyme that joins two DNA fragments by catalyzing the formation of an internucleotide ester bond between phosphate and deoxyribose. It is active during DNA replication, DNA repair and DNA recombination. There are two forms of DNA ligase: one requires ATP (EC 6.5.1.1), the other NAD (EC 6.5.1.2).

Eukaryotic, archaebacterial, virus and phage DNA ligases are ATP-dependent. During the first step of the joining reaction, the ligase interacts with ATP to form a covalent enzyme-adenylate intermediate. A conserved lysine residue is the site of adenylation [1,2].

Apart from the active site region, the only conserved region common to all ATP-dependent DNA ligases is found [3] in the C-terminal section and contains a conserved glutamate as well as four positions with conserved basic residues.

Signature patterns were developed for both conserved regions.

Consensus pattern[EDQH]-x-K-x-[DN]-G-x-R-[GACIVM] [K is the active site residue]

Consensus patternE-G-[LIVMA]-[LIVM](2)-[KR]-x(5,8)-[YW]-[QNEK]-x(2,6)-[KRH]-x(3,5)-K-[LIVMFY]-K

Sequences known to belong to this class detected by the patternALL, except for archebacterial DNA ligases.

[1]
Tomkinson A.E., Totty N.F., Ginsburg M., Lindahl T.
Proc. Natl. Acad. Sci. U.S.A. 88:400-404(1991).
[2]
Lindahl T., Barnes D.E.
Annu. Rev. Biochem. 61:251-281(1992).
[3]
Kletzin A.
Nucleic Acids Res. 20:5389-5396(1992).

812. (FAD_Gly3P_dh) FAD-dependent glycerol-3-phosphate dehydrogenase signatures
PROSITE cross-reference(s): PS00977; FAD_G3PDH_1, PS00978; FAD_G3PDH_2

FAD-dependent glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) (GPD) catalyzes the conversion of glycerol-3-phosphate into dihydroxyacetone phosphate. In bacteria [1] it is associated with the utilization of glycerol coupled to respiration. In Escherichia coli, two isozymes are known: one expressed under anaerobic conditions (gene glpA) and one in aerobic conditions (gene glpD). In eukaryotes, a mitochondrial form of GPD participates in the glycerol phosphate shuttle in conjunction with an NAD-dependent cytoplasmic GPD (EC 1.1.1.8) [2, 3].

These enzymes are proteins of about 60 to 70 Kd which contain a probable FAD-binding domain in their N-terminal extremity. The mammalian enzyme differs from the bacterial or yeast proteins by having an EF-hand calcium-binding region (See <PDOC00018>) in its C-terminal extremity.

Two signature patterns were developed. One based on the first half of the FAD-binding domain and one which corresponds to a conserved region in the central part of these enzymes.

Consensus pattern[IV]-G-G-G-x(2)-G-[STACV]-G-x-A-x-D-x(3)-R-G

Consensus patternG-G-K-x(2)-[GSTE]-Y-R-x(2)-A

[1]
Austin D., Larson T.J.
J. Bacteriol. 173:101-107(1991).
[2]
Roennow B., Kielland-Brandt M.C.
Yeast 9:1121-1130(1993).
[3]
Brown L.J., McDonald M.J., Lehn D.A., Moran S.M.
J. Biol. Chem. 269:14363-14366(1994).

813. (Fapy_DNA_glyco) Formamidopyrimidine-DNA glycosylase signature
PROSITE cross-reference(s): PS01242; FPG

Formamidopyrimidine-DNA glycosylase (EC 3.2.2.23) [1] (Fapy-DNA glycosylase) (gene fpg) is a bacterial enzyme involved in DNA repair and which excise oxidized purine bases to release 2,6-diamino-4-hydroxy-5N-methylformamido-pyrimidine (Fapy) and 7,8-dihydro-8-oxoguanine (8-OxoG) residues. In addition to its glycosylase activity, FPG can also nick DNA at apurinic/apyrimidinic sites (AP sites). FPG is a monomeric protein of about 32 Kd which binds and require zinc for its activity.

The binding site for zinc seems to be located in the C-terminal part of the enzyme where fours conserved and essential [2] cysteines are located. A signature pattern was developed based on this region.

Consensus patternC-x(2,4)-C-x-[GTAQ]-x-[IV]-x(7)-R-[GSTAN]-[STA]-x-[FYI]-C- x(2)-C-Q

[The four C's are putative zinc ligands]

[1]
Duwat P., de Oliveira R., Ehrlich S.D., Boiteux S.
Microbiology 141:411-417(1995).
[2]
O'Connor T.E., Graves R.J., Demurcia G., Castaing B., Laval J.
J. Biol. Chem. 268:9063-9070(1993).

09525507.051901

814. (G_glu_transpept) Gamma-glutamyltranspeptidase signature
PROSITE cross-reference(s): PS00462; G_GLU_TRANSPEPTIDASE

Gamma-glutamyltranspeptidase (EC 2.3.2.2) (GGT) [1] catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione. In prokaryotes and eukaryotes, it is an enzyme that consists of two polypeptide chains, a heavy and a light subunit, processed from a single chain precursor. The active site of GGT is known to be located in the light subunit.

The sequences of mammalian and bacterial GGT show a number of regions of high similarity [2]. Pseudomonas cephalosporin acylases (EC 3.5.1.-) that convert 7-beta-(4-carboxybutanamido)-cephalosporanic acid (GL-7ACA) into 7-aminocephalosporanic acid (7ACA) and glutaric acid are evolutionary related to GGT and also show some GGT activity [3]. Like GGT, these GL-7ACA acylases, are also composed of two subunits.

One of the conserved regions correspond to the N-terminal extremity of the mature light chains of these enzymes. This region was used as a signature pattern.

Consensus patternT-[STA]-H-x-[ST]-[LIVMA]-x(4)-G-[SN]-x-V-[STA]-x-T-x-T-[LIVM]-[NE]-x(1,2)-[FY]-G

[1]
Tate S.S., Meister A.
Meth. Enzymol. 113:400-419(1985).
[2]
Suzuki H., Kumagai H., Echigo T., Tochikura T.
J. Bacteriol. 171:5169-5172(1989).
[3]
Ishiye M., Niwa M.
Biochim. Biophys. Acta 1132:233-239(1992).

09555316.061701

815. G-protein gamma subunit profile
PROSITE cross-reference(s): PS50058; G_PROTEIN_GAMMA

Guanine nucleotide-binding proteins (G proteins) [1] act as intermediaries in the transduction of signals generated by transmembrane receptors. G proteins consist of three subunits (alpha, beta, and gamma). The alpha subunit binds to and hydrolyzes GTP; the functions of the beta and gamma subunits are less clear but they seem to be required for the replacement of GDP by GTP as well as for membrane anchoring and receptor recognition.

The gamma subunits are small proteins (from 70 to 110 residues) that are bound to the membrane via a isoprenyl group (either a farnesyl or a geranyl-geranyl) covalently linked to their C-terminus. In mammals there are at least 12 different isoforms of gamma subunits.

The Caenorhabditis elegans protein egl-10, which is a regulator of G-protein signalling, contains a G-protein gamma-like domain.

A profile was developed that spans the complete length of the gamma subunit.

[1]
Pennington S.R.
Protein Prof. 2:16-315(1995).

816. GNS1/SUR4 family signature
PROSITE cross-reference(s): PS01188; GNS1_SUR4

The following group of eukaryotic integral membrane proteins, whose exact function has not yet clearly been established, are evolutionary related [1]:

- Yeast GNS1 [2], a protein involved in synthesis of 1,3-beta-glucan.
- Yeast SUR4 (or APA1, SRE1) [3], a protein that could act in a glucose-signaling pathway that controls the expression of several genes that are transcriptionally regulated by glucose.
- Yeast hypothetical protein YJL196c.
- Caenorhabditis elegans hypothetical protein C40H1.4.
- Caenorhabditis elegans hypothetical protein D2024.3.

The proteins have from 290 to 435 amino acid residues. Structurally, they seem to be formed of three sections: a N-terminal region with two transmembrane domains, a central hydrophilic loop and a C-terminal region that contains from one to three transmembrane domains. A conserved region that contains three histidines was selected as a signature pattern. This region is located in the hydrophilic loop.

Consensus patternL-x-F-L-H-x-Y-H-H

[1]
Bairoch A.
Unpublished observations (1996).
[2]

El-Sherbeini M., Clemas J.A.
J. Bacteriol. 177:3227-3234(1995).
[3]
Garcia-Arranz M., Maldonado A.M., Mazon M.J., Portillo F.
J. Biol. Chem. 269:18076-18082(1994).

817. Immunoglobulins and major histocompatibility complex proteins signature
PROSITE cross-reference(s): PS00290; IG_MHC

The basic structure of immunoglobulin (Ig) [1] molecules is a tetramer of two light chains and two heavy chains linked by disulfide bonds. There are two types of light chains: kappa and lambda, each composed of a constant domain (CL) and a variable domain (VL). There are five types of heavy chains: alpha, delta, epsilon, gamma and mu, all consisting of a variable domain (VH) and three (in alpha, delta and gamma) or four (in epsilon and mu) constant domains (CH1 to CH4).

09555596.061600

The major histocompatibility complex (MHC) molecules are made of two chains. In class I [2] the alpha chain is composed of three extracellular domains, a transmembrane region and a cytoplasmic tail. The beta chain (beta-2-microglobulin) is composed of a single extracellular domain. In class II [3], both the alpha and the beta chains are composed of two extracellular domains, a transmembrane region and a cytoplasmic tail.

It is known [4,5] that the Ig constant chain domains and a single extracellular domain in each type of MHC chains are related. These homologous domains are approximately one hundred amino acids long and include a conserved intradomain disulfide bond. A small pattern around the C-terminal cysteine is involved in this disulfide bond which can be used to detect these category of Ig related proteins.

Consensus pattern[FY]-x-C-x-[VA]-x-H-Sequences known to belong to this class detected by the pattern: Ig heavy chains type Alpha C region : All, in CH2 and CH3. Ig heavy chains type Delta C region : All, in CH3. Ig heavy chains type Epsilon C region: All, in CH1, CH3 and CH4. Ig heavy chains type Gamma C region : All, in CH3 and also CH1 in some cases Ig heavy chains type Mu C region : All, in CH2, CH3 and CH4. Ig light chains type Kappa C region : In all CL except rabbit and Xenopus. Ig light chains type Lambda C region : In all CL except rabbit. MHC class I alpha chains : All, in alpha-3 domains, including in the cytomegalovirus MHC-1 homologous protein [6]. Beta-2-microglobulin : All. MHC class II alpha chains: All, in alpha-2 domains. MHC class II beta chains: All, in beta-2 domains.

[1]
Gough N.
Trends Biochem. Sci. 6:203-205(1981).
[2]
Klein J., Figueroa F.
Immunol. Today 7:41-44(1986).
[3]
Figueroa F., Klein J.
Immunol. Today 7:78-81(1986).
[4]
Orr H.T., Lancet D., Robb R.J., Lopez de Castro J.A., Strominger J.L.
Nature 282:266-270(1979).
[5]
Cushley W., Owen M.J.
Immunol. Today 4:88-92(1983).
[6]
Beck S., Barrel B.G.
Nature 331:269-272(1988).

818. (IGFBP) Insulin-like growth factor binding proteins signature
PROSITE cross-reference(s): PS00222; IGF_BINDING

The insulin-like growth factors (IGF-I and IGF-II) bind to specific binding proteins in extracellular fluids with high affinity [1,2,3]. These IGF-binding proteins (IGFBP) prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cells culture. They seem to alter the interaction of IGFs with their cell surface receptors. There are at least six different IGFBPs and they are structurally related.

The following growth-factor inducible proteins are structurally related to IGFBPs and could function as growth-factor binding proteins [4,5]:

- Mouse protein cyr61 and its probable chicken homolog, protein CEF-10.
- Human connective tissue growth factor (CTGF) and its mouse homolog, protein FISP-12.
- Vertebrate protein NOV.

09555326.051600

As a signature pattern a conserved cysteine-rich region locatedin the N-terminal section of these proteins is used.

Consensus patternG-C-[GS]-C-C-x(2)-C-A-x(6)-C
Sequences known to belong to this class detected by the patternALL, except for IGFBP-6's.

[1]
Rechler M.M.
Vitam. Horm. 47:1-114(1993).
[2]
Shimasaki S., Ling N.
Prog. Growth Factor Res. 3:243-266(1991).
[3]
Clemmons D.R.
Trends Endocrinol. Metab. 1:412-417(1990).
[4]
Bradham D.M., Igarashi A., Potter R.L., Grotendorst G.R.

J. Cell Biol. 114:1285-1294(1991).

[5]

Maloisel V., Martinerie C., Dambrine G., Plassiart G., Brisac M., Crochet J., Perbal B.

Mol. Cell. Biol. 12:10-21(1992).

819. LMWPc : Low molecular weight phosphotyrosine protein phosphatase

Number of members: 34

[1]Medline: 94329182, The crystal structure of a low-molecular-weight phosphotyrosine protein phosphatase. Su XD, Taddei N, Stefani M, Ramponi G, Nordlund P; Nature 1994;370:575-578.

09555326.071502

820. (myosin_head) ATP/GTP-binding site motif A (P-loop)

PROSITE cross-reference(s): PS00017; ATP_GTP_A

From sequence comparisons and crystallographic data analysis it has been shown [1,2,3,4,5,6] that an appreciable proportion of proteins that bind ATP or GTP share a number of more or less conserved sequence motifs. The best conserved of these motifs is a glycine-rich region, which typically forms a flexible loop between a beta-strand and an alpha-helix. This loop interacts with one of the phosphate groups of the nucleotide. This sequence motif is generally referred to as the 'A' consensus sequence [1] or the 'P-loop' [5].

There are numerous ATP- or GTP-binding proteins in which the P-loop is found. A number of protein families for which the relevance of the presence of such motif has been noted is listed below:

- ATP synthase alpha and beta subunits (see <PDOC00137>).
- Myosin heavy chains.
- Kinesin heavy chains and kinesin-like proteins (see <PDOC00343>).
- Dynamins and dynamin-like proteins (see <PDOC00362>).
- Guanylate kinase (see <PDOC00670>).

- Thymidine kinase (see <PDOC00524>).
- Thymidylate kinase (see <PDOC01034>).
- Shikimate kinase (see <PDOC00868>).
- Nitrogenase iron protein family (nifH/frxC) (see <PDOC00580>).
- ATP-binding proteins involved in 'active transport' (ABC transporters) [7] (see <PDOC00185>).
- DNA and RNA helicases [8,9,10].
- GTP-binding elongation factors (EF-Tu, EF-1alpha, EF-G, EF-2, etc.).
- Ras family of GTP-binding proteins (Ras, Rho, Rab, Ral, Ypt1, SEC4, etc.).
- Nuclear protein ran (see <PDOC00859>).
- ADP-ribosylation factors family (see <PDOC00781>).
- Bacterial dnaA protein (see <PDOC00771>).
- Bacterial recA protein (see <PDOC00131>).
- Bacterial recF protein (see <PDOC00539>).
- Guanine nucleotide-binding proteins alpha subunits (Gi, Gs, Gt, G0, etc.).
- DNA mismatch repair proteins mutS family (See <PDOC00388>).
- Bacterial type II secretion system protein E (see <PDOC00567>).

Not all ATP- or GTP-binding proteins are picked-up by this motif. A number of proteins escape detection because the structure of their ATP-binding site is completely different from that of the P-loop. Examples of such proteins are the E1-E2 ATPases or the glycolytic kinases. In other ATP- or GTP-binding proteins the flexible loop exists in a slightly different form; this is the case for tubulins or protein kinases. A special mention must be reserved for adenylate kinase, in which there is a single deviation from the P-loop pattern: in the last position Gly is found instead of Ser or Thr.

Consensus pattern[AG]-x(4)-G-K-[ST]

[1]
Walker J.E., Saraste M., Runswick M.J., Gay N.J.
EMBO J. 1:945-951(1982).
[2]

09555326.051502

Moller W., Amons R.

FEBS Lett. 186:1-7(1985).

[3]

Fry D.C., Kuby S.A., Mildvan A.S.

Proc. Natl. Acad. Sci. U.S.A. 83:907-911(1986).

[4]

Dever T.E., Glynias M.J., Merrick W.C.

Proc. Natl. Acad. Sci. U.S.A. 84:1814-1818(1987).

[5]

Saraste M., Sibbald P.R., Wittinghofer A.

Trends Biochem. Sci. 15:430-434(1990).

[6]

Koonin E.V.

J. Mol. Biol. 229:1165-1174(1993).

[7]

Higgins C.F., Hyde S.C., Mimmack M.M., Gileadi U., Gill D.R., Gallagher M.P.

J. Bioenerg. Biomembr. 22:571-592(1990).

[8]

Hodgman T.C.

Nature 333:22-23(1988) and Nature 333:578-578(1988) (Errata).

[9]

Linder P., Lasko P., Ashburner M., Leroy P., Nielsen P.J., Nishi K., Schnier J., Slonimski P.P.

Nature 337:121-122(1989).

[10]

Gorbalenya A.E., Koonin E.V., Donchenko A.P., Blinov V.M.

Nucleic Acids Res. 17:4713-4730(1989).

821. PE: PE family

This family named after a PE motif near to the amino terminus of the domain. The PE family of proteins all contain an amino-terminal region of about 110 amino acids. The carboxyl terminus of this family are variable and fall into several classes. The largest class of PE proteins is the highly repetitive PGRS class which have a high glycine content. The function of these proteins is uncertain but it has been suggested that they may be related to antigenic variation of Mycobacterium tuberculosis [1]. Number of members: 88

[1] Medline: 98295987. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Barrell BG, et al; Nature 1998;393:537-544.

822. (RNB) Ribonuclease II family signature
PROSITE cross-reference(s): PS01175; RIBONUCLEASE_II

09959325.061801

On the basis of sequence similarities, the following bacterial and eukaryotic proteins seem to form a family:

- Escherichia coli and related bacteria ribonuclease II (EC 3.1.13.1) (RNase II) (gene rnb) [1]. RNase II is an exonuclease involved in mRNA decay. It degrades mRNA by hydrolyzing single-stranded polyribonucleotides processively in the 3' to 5' direction.
- Bacterial protein vacB. In Shigella flexneri, vacB has been shown to be required for the expression of virulence genes at the posttranscriptional level.
- Yeast protein SSD1 (or SRK1) which is implicated in the control of the cell cycle G1 phase.
- Yeast protein DIS3 [2], which binds to ran (GSP1) and ehances the the nucleotide-releasing activity of RCC1 on ran.
- Fission yeast protein dis3, which is implicated in mitotic control.
- Neurospora crassa cyt-4, a mitochondrial protein required for RNA 5' and 3' end processing and splicing.
- Yeast protein MSU1, which is involved in mitochondrial biogenesis.
- Synechocystis strain PCC 6803 protein zam [3], which control resistance to the carbonic anhydrase inhibitor acetazolamide.

- Caenorhabditis elegans hypothetical protein F48E8.6.

The size of these proteins range from 644 residues (rnb) to 1250 (SSD1). While their sequence is highly divergent they share a conserved domain in their C-terminal section [4]. It is possible that this domain plays a role in a putative exonuclease function that would be common to all these proteins. A signature pattern was developed based on the core of this conserved domain.

Consensus pattern[HI]-[FYE]-[GSTAM]-[LIVM]-x(4,5)-Y-[STAL]-x-[FWVAC]-[TV]-[SA]-P-[LIVMA]-[RQ]-[KR]-[FY]-x-D-x(3)-[HQ]

[1]
Zilhao R., Camelo L., Arraiano C.M.
Mol. Microbiol. 8:43-51(1993).
[2]
Noguchi E., Hayashi N., Azuma Y., Seki T., Nakamura M., Nakashima N.,
Yanagida M., He X., Mueller U., Sazer S., Nishimoto T.
EMBO J. 15:5595-5605(1996).
[3]
Beuf L., Bedu S., Cami B., Joset F.
Plant Mol. Biol. 27:779-788(1995).
[4]
Mian I.S.
Nucleic Acids Res. 25:3187-3195(1997).

09559356.061502

823. Src homology 2 (SH2) domain profile
PROSITE cross-reference(s): PS50001; SH2

The Src homology 2 (SH2) domain is a protein domain of about 100 amino-acid residues first identified as a conserved sequence region between the oncoproteins Src and Fps [1]. Similar sequences were later found in many other intracellular signal-transducing proteins [2]. SH2 domains function as regulatory modules of intracellular signalling cascades by interacting with high affinity to phosphotyrosine-containing target peptides in a sequence-specific and strictly phosphorylation-dependent manner [3,4,5,6].

The SH2 domain has a conserved 3D structure consisting of two alpha helices and six to seven beta-strands. The core of the domain is formed by a continuous beta-meander composed of two connected beta-sheets [7].

So far, SH2 domains have been identified in the following proteins:

- Many vertebrate, invertebrate and retroviral cytoplasmic (non-receptor) protein tyrosine kinases. In particular in the Src, Abl, Bkt, Csk and ZAP70 families of kinases.
- Mammalian phosphatidylinositol-specific phospholipase C gamma-1 and -2. Two copies of the SH2 domain are found in those proteins in between the catalytic 'X-' and 'Y-boxes' (see <PDOC50007>).
- Mammalian phosphatidyl inositol 3-kinase regulatory p85 subunit.
- Some vertebrate and invertebrate protein-tyrosine phosphatases.
- Mammalian Ras GTPase-activating protein (GAP).
- Adaptor proteins mediating binding of guanine nucleotide exchange factors to growth factor receptors: vertebrate GRB2, Caenorhabditis elegans sem-5 and Drosophila DRK.
- Mammalian Vav oncoprotein, a guanine-nucleotide exchange factor of the CDC24 family.
- Miscellanous proteins interacting with vertebrate receptor protein tyrosine kinases: oncoprotein Crk, mammalian cytoplasmic proteins Nck, Shc.
- STAT proteins (signal transducers and activators of transcription).
- Chicken tensin.
- Yeast transcriptional control protein SPT6.

The profile developed to detect SH2 domains is based on a structural alignment consisting of 8 gap-free blocks and 7 linker regions totaling 92 match positions.

09535261.010702

[1]
Sadowski I., Stone J.C., Pawson T.
Mol. Cell. Biol. 6:4396-4408(1986).
[2]
Russel R.B., Breed J., Barton G.J.
FEBS Lett. 304:15-20(1992).
[3]
Marangere L.E.M., Pawson T.
J. Cell Sci. Suppl. 18:97-104(1994).
[4]
Pawson T., Schlessinger J.
Curr. Biol. 3:434-442(1993).
[5]
Mayer B.J., Baltimore D.
Trends Cell. Biol. 3:8-13(1993).
[6]
Pawson T.
Nature 373:573-580(1995).
[7]
Kuriyan J., Cowburn D.
Curr. Opin. Struct. Biol. 3:828-837(1993).

09525325.061502

824. Sulfate transporters signature
PROSITE cross-reference(s): PS01130; SULFATE_TRANSP

A number of proteins involved in the transport of sulfate across a membrane as well as some yet uncharacterized proteins have been shown [1,2] to be evolutionary related. These proteins are:

- Neurospora crassa sulfate permease II (gene cys-14).
- Yeast sulfate permeases (genes SUL1 and SUL2).
- Rat sulfate anion transporter 1 (SAT-1).
- Mammalian DTDST, a probable sulfate transporter which, in Human, is

involved in the genetic disease, diastrophic dysplasia (DTD).
- Sulfate transporters 1, 2 and 3 from the legume Stylosanthes hamata.

- Human pendrin (gene PDS), which is involved in a number of hearing loss genetic diseases.
- Human protein DRA (Down-Regulated in Adenoma).
- Soybean early nodulin 70.
- Escherichia coli hypothetical protein ychM.
- Caenorhabditis elegans hypothetical protein F41D9.5.

As expected by their transport function, these proteins are highly hydrophobic and seem to contain about 12 transmembrane domains. The best conserved region seems to be located in the second transmembrane region and is used as a signature pattern.

Consensus pattern[PAV]-x-Y-[GS]-L-Y-[STAG](2)-x(4)-[LIVFYA]-[LIVST]-[YI]-x(3)-[GA]-[GST]-S-[KR]

[1]
Sandal N.N., Marcker K.A.
Trends Biochem. Sci. 19:19-19(1994).
[2]
Smith F.W., Hawkesford M.J., Prosser I.M., Clarkson D.T.
Mol. Gen. Genet. 247:709-715(1995).

825. TYA: TYA transposon protein

Ty are yeast transposons. A 5.7kb transcript codes for p3 a fusion protein of TYA and TYB. The TYA protein is analogous to the gag protein of retroviruses. TYA a is cleaved to form 46kd protein which can form mature virion like particles [1]. Number of members: 59

[1] Medline: 97404699. Cryo-electron microscopy structure of yeast Ty retrotransposon virus-like particles. Palmer KJ, Tichelaar W, Myers N, Burns NR, Butcher SJ, Kingsman AJ, Fuller SD, Saibil HR; J Virol 1997;71:6863-6868.

09555916.061900

826. Aldolase_II

Class II Aldolase and Adducin N-terminal domain.

-!- This family includes class II aldolases and adducins which have not been ascribed any enzymatic function. Number of members: 37

References:

[1] Medline: 93294819. The spatial structure of the class II L-fuculose-1-phosphate aldolase from Escherichia coli. Dreyer MK, Schulz GE; J Mol Biol 1993;231:549-553.

[2] Medline: 96256522. Catalytic mechanism of the metal-dependent fuculose aldolase from Escherichia coli as derived from the structure. Dreyer MK, Schulz GE; J Mol Biol 1996;259:458-466.

827. CBD_2

-!- Two tryptophan residues are involved in cellulose binding.

-!- Cellulose binding domain found in bacteria. Number of members: 51

References:

[1] Medline: 95284032. Solution structure of a cellulose-binding domain from Cellulomonas fimi by nuclear magnetic resonance spectroscopy. Xu GY, Ong E, Gilkes NR, Kilburn DG, Muhandiram DR, Harris-Brandts M, Carver JP, Kay LE, Harvey TS; Biochemistry 1995;34:6993-7009.

828. P

A unique feature of the eukaryotic subtilisin-like proprotein convertases is the presence of an additional highly conserved sequence of approximately 150 residues (P domain) located immediately downstream of the catalytic domain.

Number of members: 91

References:

[1] Medline: 94252314. A C-terminal domain conserved in precursor processing proteases is required for intramolecular N-terminal maturation of pro-Kex2 protease. Gluschankof P, Fuller RS; EMBO J 1994;13:2280-2288.

[2] Medline: 98225190. Regulatory roles of the P domain of the subtilisin-like prohormone convertases. Zhou A, Martin S, Lipkind G, LaMendola J, Steiner DF; J Biol Chem 1998;273:11107-11114.

829. Uncharacterized protein family UPF0020 signature

PROSITE cross-reference(s): PS01261; UPF0020

The following uncharacterized proteins have been shown [1] to share regions of similarities:

- Escherichia coli hypothetical protein ycbY and HI0116/15, the corresponding Haemophilus influenzae protein.
- Bacillus subtilis hypothetical protein ypsC.
- Synechocystis strain PCC 6803 hypothetical protein slr0064.
- Methanococcus jannaschii hypothetical proteins MJ0438 and MJ0710.

These are hydrophilic proteins of from 40 Kd to about 80 Kd. They can be picked up in the database by the following pattern.

Consensus patternD-P-[LIVMF]-C-G-[ST]-G-x(3)-[LI]-E

References:

[1] Bairoch A. Unpublished observations (1997).

830. Uncharacterized protein family UPF0031 signatures

PROSITE cross-reference(s): PS01049; UPF0031_1; PS01050; UPF0031_2

The following uncharacterized proteins have been shown [1] to share regions of similarities:

- Yeast chromosome XI hypothetical protein YKL151c.
- Caenorhabditis elegans hypothetical protein R107.2.
- Escherichia coli hypothetical protein yjeF.
- Bacillus subtilis hypothetical protein yxkO.
- Helicobacter pylori hypothetical protein HP1363.

- Mycobacterium tuberculosis hypothetical protein MtCY77.05c.
- Mycobacterium leprae hypothetical protein B229_C2_201.
- Synechocystis strain PCC 6803 hypothetical protein sll1433.
- Methanococcus jannaschii hypothetical protein MJ1586.

These are proteins of about 30 to 40 Kd whose central region is well conserved. They can be picked up in the database by the following patterns.

Consensus pattern[SAV]-[IVW]-[LVA]-[LIV]-G-[PNS]-G-L-[GP]-x-[DENQT]
Consensus pattern[GA]-G-x-G-D-[TV]-[LT]-[STA]-G-x-[LIVM]

09535205.052502

831. (ACOX)
Acyl-CoA oxidase

This is a family of Acyl-CoA oxidases EC:1.3.3.6. Acyl-coA oxidase converts acyl-CoA into trans-2-enoyl-CoA [1].

Number of members: 39

[1] Hayashi H, De Bellis L, Yamaguchi K, Kato A, Hayashi M, Nishimura M; Medline: 98192624. “Molecular characterization of a glyoxysomal long chain acyl-CoA oxidase that is synthesized as a precursor of higher molecular mass in pumpkin.” J Biol Chem 1998;273:8301-8307.

832. (AICARFT_IMPCHas)
AICARFT/IMPCHase bienzyme

This is a family of bifunctional enzymes catalysing the last steps in de novo purine biosynthesis. The bifunctional enzyme is found in both prokaryotes and eukaryotes. The second last step is catalysed by 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase EC:2.1.2.3 (AICARFT), this enzyme catalyses the formylation of AICAR with 10-formyl-tetrahydrofolate to yield FAICAR and tetrahydrofolate [1]. The last step is catalysed by IMP (Inosine monophosphate) cyclohydrolase EC:3.5.4.10 (IMPCHase), cyclizing FAICAR (5-formylaminoimidazole-4-carboxamide ribonucleotide) to IMP [1].

Number of members: 22

[1] Akira T, Komatsu M, Nango R, Tomooka A, Konaka K, Yamauchi M, Kitamura Y, Nomura S, Tsukamoto I; Medline: 97473523 “Molecular cloning and expression of a rat cDNA encoding 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase” [published erratum appears in Gene 1998 Feb 27;208(2):337] Gene 1997;197:289-293.

[2] Rayl EA, Moroson BA, Beardsley GP; Medline: 96147205 “The human purH gene product, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase. Cloning, sequencing, expression, purification, kinetic analysis, and domain mapping.” J Biol Chem 1996;271:2225-2233.

09555326.061502

833. (AOX)

Alternative oxidase

The alternative oxidase is used as a second terminal oxidase in the mitochondria, electrons are transfered directly from reduced ubiquinol to oxygen forming water [2]. This is not coupled to ATP synthesis and is not inhibited by cyanide, this pathway is a single step process [1]. In rice the transcript levels of the alternative oxidase are increased by low temperature [1].

Number of members: 27

[1] Ito Y, Saisho D, Nakazono M, Tsutsumi N, Hirai A; Medline: 98086211 “Transcript levels of tandem-arranged alternative oxidase genes in rice are increased by low temperature.” Gene 1997;203:121-129.

[2] Li Q, Ritzel RG, McLean LL, McIntosh L, Ko T, Bertrand H, Nargang FE; Medline: 96366413 “Cloning and analysis of the alternative oxidase gene of Neurospora crassa.” Genetics 1996;142:129-140.

834. (APH)

Protein kinases signatures and profile

Cross-reference(s): PS00107; PROTEIN_KINASE_ATP, PS00108; PROTEIN_KINASE_ST, PS00109; PROTEIN_KINASE_TYR, PS50011; PROTEIN_KINASE_DOM

Eukaryotic protein kinases [1 to 5] are enzymes that belong to a very extensive family of proteins which share a conserved catalytic core common to both serine/threonine and tyrosine protein kinases. There are a number of conserved regions in the catalytic domain of protein kinases. Two of these regions have been selected to build signature patterns. The first region, which is located in the N-terminal extremity of the catalytic domain, is a glycine-rich stretch of residues in the vicinity of a lysine residue, which has been shown to be involved in ATP binding. The second region, which is located in the central part of the catalytic domain, contains a conserved aspartic acid residue which is important for the catalytic activity of the enzyme [6]; two signature patterns were derived for that region: one specific for serine/ threonine kinases and the other for tyrosine kinases. A profile was developed which is based on the alignment in [1] and covers the entire catalytic domain.

Consensus pattern: [LIV]-G-{P}-G-{P}-[FYWMGSTNH]-[SGA]-{PW}-[LIVCAT]-{PD}-x-[GSTACLIVMFY]-x(5,18)-[LIVMFYWCSTAR]-[AIVP]-[LIVMFAGCKR]-K [K binds ATP]

Sequences known to belong to this class detected by the pattern the majority of known protein kinases but it fails to find a number of them, especially viral kinases which are quite divergent in this region and are completely missed by this pattern.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3) [D is an active site residue]

Sequences known to belong to this class detected by the pattern. Most serine/ threonine specific protein kinases with 10 exceptions (half of them viral kinases) and also Epstein-Barr virus BGLF4 and Drosophila ninaC which have respectively Ser and Arg instead of the conserved Lys and which are therefore detected by the tyrosine kinase specific pattern described below.

Consensus pattern: [LIVMFYC]-x-[HY]-x-D-[LIVMFY]-[RSTAC]-x(2)-N-[LIVMFYC](3) [D is an active site residue] tyrosine specific protein kinases with the exception of human ERBB3 and mouse blk. This pattern will also detect most bacterial aminoglycoside phosphotransferases [8,9] and herpesviruses ganciclovir kinases [10]; which are proteins structurally and evolutionary related to protein kinases. Sequences known to belong to this class detected by the profile ALL, except for three viral kinases. This profile also detects receptor guanylate cyclases (see <PDOC00430>) and 2-5A-dependent ribonucleases. Sequence similarities between these two families and the eukaryotic protein kinase family have been noticed before. It also detects Arabidopsis thaliana kinase- like protein TMKL1 which seems to have lost its catalytic activity.

09595526.052401

Note if a protein analyzed includes the two protein kinase signatures, the probability of it being a protein kinase is close to 100%. Note eukaryotic-type protein kinases have also been found in prokaryotes such as Myxococcus xanthus [11] and Yersinia pseudotuberculosis. Note the patterns shown above has been updated since their publication in [7]. Note this documentation entry is linked to both signature patterns and a profile. As the profile is much more sensitive than the patterns, you should use it if you have access to the necessary software tools to do so.

References

[1] Hanks S.K., Hunter T., FASEB J. 9:576-596(1995).
[2] Hunter T., Meth. Enzymol. 200:3-37(1991).
[3] Hanks S.K., Quinn A.M., Meth. Enzymol. 200:38-62(1991).
[4] Hanks S.K., Curr. Opin. Struct. Biol. 1:369-383(1991).

[5] Hanks S.K., Quinn A.M., Hunter T., Science 241:42-52(1988).

[6] Knighton D.R., Zheng J., Ten Eyck L.F., Ashford V.A., Xuong N.-H., Taylor, S.S., Sowadski J.M., Science 253:407-414(1991).

[7] Bairoch A., Claverie J.-M., Nature 331:22(1988).

[8] Benner S., Nature 329:21-21(1987).

[9] Kirby R., J. Mol. Evol. 30:489-492(1992).

[10] Littler E., Stuart A.D., Chee M.S., Nature 358:160-162(1992).

[11] Munoz-Dorado J., Inouye S., Inouye M., Cell 67:995-1006(1991).

835. (Asp_Glu_race)

Aspartate and glutamate racemases signatures

Cross-reference(s) PS00923; ASP_GLU_RACEMASE_1 PS00924; ASP_GLU_RACEMASE_2

Aspartate racemase (EC 5.1.1.13) and glutamate racemase (EC 5.1.1.3) are two evolutionary related bacterial enzymes that do not seem to require a cofactor for their activity [1]. Glutamate racemase, which interconverts L-glutamate into D-glutamate, is required for the biosynthesis of peptidoglycan and some peptide-based antibiotics such as gramicidin S. In addition to characterized aspartate and glutamate racemases, this family also includes a hypothetical protein from Erwinia carotovora and one from Escherichia coli (ygeA). Two conserved cysteines are present in the sequence of these enzymes. They are expected to play a role in catalytic activity by acting as bases in proton abstraction from the substrate. Signature patterns were developed for both cysteines.

Consensus pattern: [IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK]

Consensus pattern: [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM]

[1] Gallo K.A., Knowles J.R., Biochemistry 32:3981-3990(1993).

09555355.051500

836. (ATP-sulfurylase)
ATP-sulfurylase

This family consists of ATP-sulfurylase or sulfate adenylyltransferase EC:2.7.7.4 some of which are part of a bifunctional polypeptide chain associated with adenosyl phosphosulphate (APS) kinase APS_kinase. Both enzymes are required for PAPS (phosphoadenosine-phosphosulfate) synthesis from inorganic sulphate [2]. ATP sulfurylase catalyses the synthesis of adenosine-phosphosulfate APS from ATP and inorganic sulphate [1].

Number of members: 37

[1] Kurima K, Warman ML, Krishnan S, Domowicz M, Krueger RC Jr, Deyrup A, Schwartz NB; Medline: 98337975 “A member of a family of sulfate-activating enzymes causes murine brachymorphism” [published erratum appears in Proc Natl Acad Sci U S A 1998 Sep 29;95(20):12071] Proc Natl Acad Sci U S A 1998;95:8681-8685.

[2] Rosenthal E, Leustek T; Medline: 96096529 “A multifunctional Urechis caupo protein, PAPS synthetase, has both ATP sulfurylase and APS kinase activities.” Gene 1995;165:243-248.

09535620 . 051600

837. (ATP-synt_F)
ATP synthase (F/14-kDa) subunit

This family includes 14-kDa subunit from vATPases [1], which is in the peripheral catalytic part of the complex [2]. The family also includes archaebacterial ATP synthase subunit F [3].

Number of members: 23

[1] Guo Y, Kaiser K, Wieczorek H, Dow JA; Medline: 96269411 “The Drosophila melanogaster gene vha14 encoding a 14-kDa F-subunit of the vacuolar ATPase.” Gene 1996;172:239-243.

[2] Peng SB, Crider BP, Tsai SJ, Xie XS, Stone DK; Medline: 96216416 “Identification of a 14-kDa subunit associated with the catalytic sector of clathrin-coated vesicle H+-ATPase.” J Biol Chem 1996;271:3324-3327.

[3] Wilms R, Freiberg C, Wegerle E, Meier I, Mayer F, Muller V; Medline: 96324968 “Subunit structure and organization of the genes of the A1A0 ATPase from the Archaeon Methanosarcina mazei Go1.” J Biol Chem 1996;271:18843-18852.

838. (CBD_4)

Starch binding domain

Number of members: 48

09553256.020

839. (CbiX)

The function of CbiX is uncertain, however it is found in cobalamin biosynthesis operons and so may have a related function. Some CbiX proteins contain a striking histidine-rich region at their C-terminus, which suggests that it might be involved in metal chelation [1].

Number of members: 6

[1] Raux E, Lanois A, Warren MJ, Rambach A, Thermes C; Medline: 98416126 “Cobalamin (vitamin B12) biosynthesis: identification and characterization of a Bacillus megaterium cobI operon.” Biochem J 1998;335:159-166.

840. (Complex1_51K)

Respiratory-chain NADH dehydrogenase 51 Kd subunit signatures Cross-reference(s) PS00644; COMPLEX1_51K_1 PS00645; COMPLEX1_51K_2

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 51 Kd (in mammals), which is the second largest subunit of complex I and is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind to NAD, FMN, and a 2Fe-2S cluster.

The 51 Kd subunit is highly similar to [3,4]:

- Subunit alpha of Alcaligenes eutrophus NAD-reducing hydrogenase (gene hoxF) which also binds to NAD, FMN, and a 2Fe-2S cluster.
- Subunit NQO1 of Paracoccus denitrificans NADH-ubiquinone oxidoreductase.
- Subunit F of Escherichia coli NADH-ubiquinone oxidoreductase (gene nuoF).

The 51 Kd subunit and the bacterial hydrogenase alpha subunit contains three regions of sequence similarities. The first one most probably corresponds to the NAD-binding site, the second to the FMN-binding site, and the third one, which contains three cysteines, to the iron-sulfur binding region. Signature patterns have been developed for the FMN-binding and for the 2Fe-2S binding regions.

Consensus pattern: G-[AM]-G-[AR]-Y-[LIVM]-C-G-[DE](2)-[STA](2)-[LIM](2)-[EN]- S
Consensus pattern: E-S-C-G-x-C-x-P-C-R-x-G [The three C's are putative 2Fe-2S ligands]

[1] Ragan C.I., Curr. Top. Bioenerg. 15:1-36(1987).
[2] Weiss H., Friedrich T., Hofhaus G., Preis D., Eur. J. Biochem. 197:563-576(1991).
[3] Fearnley I.M., Walker J.E. Biochim. Biophys. Acta 1140:105-134(1992).
[4] Weidner U., Geier S., Ptock A., Friedrich T., Leif H., Weiss H., J. Mol. Biol. 233:109-122(1993).

841. (DAP_epimerase)
Diaminopimelate epimerase signature

Cross-reference(s) PS01326; DAP_EPIMERASE

Diaminopimelate epimerase (EC 5.1.1.7) catalyzes the isomeriazation of L,L- to D,L-meso-diaminopimelate in the biosynthetic pathway leading from aspartate to lysine. This enzyme is a protein of about 30 Kd. Two conserved cysteines seem [1] to function as the acid and base in the catalytic mechanism. As a signature pattern, the region surrounding the first of these two active site cysteines were selected.

Consensus pattern: N-x-D-G-S-x(4)-C-G-N-[GA]-x-R [C is an active site residue] Sequences known to belong to this class detected by the pattern ALL, except for an Anabaena dapF which has a Ser instead of the active site Cys.

[1] Cirilli M., Zheng R., Scapin G., Blanchard J.S., Biochemistry 37:16452-16458(1998).

842. (DNA_gyraseB_C)

DNA topoisomerase II signature

Cross-reference(s) PS00177; TOPOISOMERASE_II

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break. Topoisomerase II is found in phages, archaebacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits (the product of genes 39, 52 and 60). In prokaryotes and in archaebacteria the enzyme, known as DNA gyrase, consists of two subunits (genes gyrA and gyrB [E2]). In some bacteria, a second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes parC and parE). In eukaryotes, type II topoisomerase is a homodimer.

There are many regions of sequence homology between the different subtypes of topoisomerase II. The relation between the different subunits is shown in the following representation:

<----------------About-1400-residues---------------------->

[----------Protein 39-*-----][----Protein 52----]   Phage T4
[----------gyrB-------*-----][--------gyrA-----------------] Prokaryote II
Archaebacteria
[----------parE-------*-----][--------parD-----------------] Prokaryote IV
[-------------------*----------------------------------] Eukaryote and
ASF

'*': Position of the pattern.

As a signature pattern for this family of proteins, a region that contains a highly conserved pentapeptide was selected. The pattern is located in gyrB, in parE, and in protein 39 of phage T4 topoisomerase.

Consensus pattern: [LIVMA]-x-E-G-[DN]-S-A-x-[STAG]

[1] Sternglanz R., Curr. Opin. Cell Biol. 1:533-535(1990).
[2] Bjornsti M.-A., Curr. Opin. Struct. Biol. 1:99-103(1991).
[3] Sharma A., Mondragon A., Curr. Opin. Struct. Biol. 5:39-47(1995).
[4] Roca J., Trends Biochem. Sci. 20:156-160(1995).

843. (DUF16)
Protein of unknown function

The function of this protein is unknown. It appears to only occur in Mycoplasma pneumoniae.

Number of members: 26

[1] Himmelreich R, Hilbert H, Plagens H, Pirkl E, Li BC, Herrmann R; Medline: 97105885
“Complete sequence analysis of the genome of the bacterium Mycoplasma pneumoniae.”
Nucleic Acids Res 1996;24:4420-4449.

844. (DUF21)

Domain of unknown function

This transmembrane region has no known function. Many of the sequences in this family are annotated as hemolysins, however this is due to a similarity to Swiss:Q54318 that does not contain this domain. This domain is found in the N-terminus of the proteins adjacent to two intracellular CBS domains CBS.

Number of members: 42

09555326.061500

845. (DUF56)

Integral membrane protein

The members of this family are putative integral membrane proteins. The function of the family is unknown, however the family includes Sec59 from yeast. Sec59 is a dolichol kinase EC:2.7.1.108, but it is not clear if the enzymatic activity resides in this region or its N terminal region.

Number of members: 13

846. (DUF94)

Domain of unknown function

The function of this domain is unknown. It is found in both eukaryotes and archaebacteria. The alignment contains a completely conserved aspartate residue that may be functionally important. The eukaryotic domains contains three conserved cysteines and a histidine that might be metal binding, however these are absent in the archaebacterial proteins.

Number of members: 9

847. (FF)

FF domain

This domain may be involved in protein-protein interaction [1].

Number of members: 42

[1] Bedford MT, Leder P; Medline: 99322199 "The FF domain: a novel motif that often accompanies WW domains." Trends Biochem Sci 1999;24:264-265.

09585306.051500

848. (FLO_LFY)

Floricaula / Leafy protein

This family consists of various plant development proteins which are homologues of floricaula (FLO) and Leafy (LFY) proteins which are floral meristem identity proteins. Mutations in the sequences of these proteins affect flower and leaf development.

Number of members: 16

[1] Hofer J, Turner L, Hellens R, Ambrose M, Matthews P, Michael A, Ellis N; Medline: 97411151 "UNIFOLIATA regulates leaf and flower morphogenesis in pea." Curr Biol 1997;7:581-587.

[2] Weigel D, Alvarez J, Smyth DR, Yanofsky MF, Meyerowitz EM; Medline: 92274452 "LEAFY controls floral meristem identity in Arabidopsis." Cell 1992;69:843-859.

849. (G-patch)

G-patch domain

This domain is found in a number of RNA binding proteins, and is also found in proteins that contain RNA binding domains. This suggests that this domain may have an RNA binding function. This domain has seven highly conserved glycines.

Number of members: 47

[1] Aravind L, Koonin EV; Medline: 10470032 “G-patch: a new conserved domain in eukaryotic RNA-processing proteins and type D retroviral polyproteins.” Trends Biochem Sci 1999;24:342-344.

850. (Gram-ve_porins)

General diffusion Gram-negative porins signature

Cross-reference(s) PS00576; GRAM_NEG_PORIN

The outer membrane of Gram-negative bacteria acts as a molecular filter for hydrophilic compounds. Proteins, known as porins [1], are responsible for the 'molecular sieve' properties of the outer membrane. Porins form large water- filled channels which allows the diffusion of hydrophilic molecules into the periplasmic space. Some porins form general diffusion channels that allows any solutes up to a certain size (that size is known as the exclusion limit) to cross the membrane, while other porins are specific for a solute and contain a binding site for that solute inside the pores (these are known as selective porins). As porins are the major outer membrane proteins, they also serve as receptor sites for the binding of phages and bacteriocins. General diffusion porins generally assemble as trimer in the membrane and the transmembrane core of these proteins is composed exclusively of beta strands [2]. It has been shown [3] that a number of general porins are evolutionary related, these porins are:

- Enterobacteria phoE.
- Enterobacteria ompC.
- Enterobacteria ompF.

- Enterobacteria nmpC.
- Bacteriophage PA-2 LC.
- Neisseria PI.A.
- Neisseria PI.B.

As a signature pattern a conserved region was selected, located in the C-terminal part of these proteins, which spans two putative transmembrane beta strands.

Consensus pattern: [LIVMFY]-x(2)-G-x(2)-Y-x-F-x-K-x(2)-[SN]-[STAV]-[LIVMFYW]- V

[1] Benz R., Bauer K., Eur. J. Biochem. 176:1-19(1988).
[2] Jap B.K., Walian P.J., Q. Rev. Biophys. 23:367-403(1990).
[3] Jeanteur D., Lakey J.H., Pattus F., Mol. Microbiol. 5:2153-2164(1991).

851. (HlyD)
HlyD family secretion proteins signature

Cross-reference(s) PS00543; HLYD_FAMILY

Gram-negative bacteria produce a number of proteins which are secreted into the growth medium by a mechanism that does not require a cleaved N-terminal signal sequence. These proteins, while having different functions, require the help of two or more proteins for their secretion across the cell envelope. Amongst which a protein belonging to the ABC transporters family (see the relevant entry <PDOC00185>) and a protein belonging to a family which is currently composed [1 to 5] of the following members:

Gene	Species	Protein which is exported
hlyD	Escherichia coli	Hemolysin
appD	A.pleuropneumoniae	Hemolysin
lcnD	Lactococcus lactis	Lactococcin A
lktD	A.actinomycetemcomitans	Leukotoxin
	Pasteurella haemolytica	
rtxD	A.pleuropneumoniae	Toxin-III

cyaD	Bordetella pertussis	Calmodulin-sensitive adenylate cyclase-hemolysin (cyclolysin)
cvaA	Escherichia coli	Colicin V
prtE	Erwinia chrysanthemi	Extracellular proteases B and C
aprE	Pseudomonas aeruginosa	Alkaline protease
emrA	Escherichia coli	Drugs and toxins
yjcR	Escherichia coli	Unknown

These proteins are evolutionary related and consist of from 390 to 480 amino acid residues. They seem to be anchored in the inner membrane by a N-terminal transmembrane region. Their exact role in the secretion process is not yet known. The C-terminal section of these proteins is the best conserved region; a signature pattern from that region was derived.

Consensus pattern: [LIVM]-x(2)-G-[LM]-x(3)-[STGAV]-x-[LIVMT]-x-[LIVMT]-[GE]-x-[KR]-x-[LIVMFYW](2)-x-[LIVMFYW](3)

Sequences known to belong to this class detected by the pattern ALL, except for emrA and yjcR.

References:

[1] Gilson L., Mahanty H.K., Kolter R., EMBO J. 9:3875-3884(1990).

[2] Letoffe S., Delepelaire P., Wandersman C., EMBO J. 9:1375-1382(1990).

[3] Stoddard G.W., Petzel J.P., van Belkum M.J., Kok J., McKay L.L., Appl. Environ. Microbiol. 58:1952-1961(1992).

[4] Duong F., Lazdunski A., Cami B., Murgier M., Gene 121:47-54(1992).

[5] Lewis K., Trends Biochem. Sci. 19:119-123(1994).

852. (IBR)

In Between Ring fingers

The IBR (In Between Ring fingers) domain is found to occur between pairs of ring fingers (zf-C3HC4). The function of this domain is unknown. This domain has also been called the C6HC domain and DRIL (for double RING finger linked) domain [2].

Number of members: 25

[1] Morett E, Bork P; Medline: 10366851 “A novel transactivation domain in parkin.”Trends Biochem Sci 1999;24:229-231.

[2] van der Reijden BA, Erpelinck-Verschueren CA, Lowenberg B, Jansen JH; Medline: 99349709 “TRIADs: a new class of proteins with a novel cysteine-rich signature.” Protein Sci 1999;8:1557-1561.

853. (IPPT)

IPP transferase

[1] Durand JM, Bjork GR, Kuwae A, Yoshikawa M, Sasakawa C; Medline: 97440126 “The modified nucleoside 2-methylthio-N6-isopentenyladenosine in tRNA of Shigella flexneri is required for expression of virulence genes.” J Bacteriol 1997;179:5777-5782.

[2] Boguta M, Hunter LA, Shen WC, Gillman EC, Martin NC, Hopper AK; Medline: 94187700 “Subcellular locations of MOD5 proteins: mapping of sequences sufficient for targeting to mitochondria and demonstration that mitochondrial and nuclear isoforms commingle in the cytosol.” Mol Cell Biol 1994;14:2298-2306.

[3] Gillman EC, Slusher LB, Martin NC, Hopper AK; Medline: 91203856 “MOD5 translation initiation sites determine N6-isopentenyladenosine modification of mitochondrial and cytoplasmic tRNA.” Mol Cell Biol 1991;11:2382-2390.

854. (KE2)

KE2 family protein

The function of members of this family is unknown, although they have been suggested to contain a DNA binding leucine zipper motif [2].

Number of members: 9

[1] Ha H, Abe K, Artzt K; Medline: 92084131 “Primary structure of the embryo-expressed gene KE2 from the mouse H-2K region.” Gene 1991;107:345-346.

[2] Shang HS, Wong SM, Tan HM, Wu M; Medline: 95129859 “YKE2, a yeast nuclear gene encoding a protein showing homology to mouse KE2 and containing a putative leucine-zipper motif.” Gene 1994;151:197-201.

855. (Lipoprotein_6)

Prokaryotic membrane lipoprotein lipid attachment site

Cross-reference(s) PS00013; PROKAR_LIPOPROTEIN

In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which a glyceride-fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]):

- Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp).
- Escherichia coli lipoprotein-28 (gene nlpA).
- Escherichia coli lipoprotein-34 (gene nlpB).
- Escherichia coli lipoprotein nlpC.
- Escherichia coli lipoprotein nlpD.
- Escherichia coli osmotically inducible lipoprotein B (gene osmB).
- Escherichia coli osmotically inducible lipoprotein E (gene osmE).
- Escherichia coli peptidoglycan-associated lipoprotein (gene pal).
- Escherichia coli rare lipoproteins A and B (genes rplA and rplB).
- Escherichia coli copper homeostasis protein cutF (or nlpE).
- Escherichia coli plasmids traT proteins.
- Escherichia coli Col plasmids lysis proteins.
- A number of Bacillus beta-lactamases.
- Bacillus subtilis periplasmic oligopeptide-binding protein (gene oppA).
- Borrelia burgdorferi outer surface proteins A and B (genes ospA and ospB).
- Borrelia hermsii variable major protein 21 (gene vmp21) and 7 (gene vmp7).
- Chlamydia trachomatis outer membrane protein 3 (gene omp3).
- Fibrobacter succinogenes endoglucanase cel-3.
- Haemophilus influenzae proteins Pal and Pcp.

- Klebsiella pullulunase (gene pulA).
- Klebsiella pullulunase secretion protein pulS.
- Mycoplasma hyorhinis protein p37.
- Mycoplasma hyorhinis variant surface antigens A, B, and C (genes vlpABC).
- Neisseria outer membrane protein H.8.
- Pseudomonas aeruginosa lipopeptide (gene lppL).
- Pseudomonas solanacearum endoglucanase egl.
- Rhodopseudomonas viridis reaction center cytochrome subunit (gene cytC).
- Rickettsia 17 Kd antigen.
- Shigella flexneri invasion plasmid proteins mxiJ and mxiM.
- Streptococcus pneumoniae oligopeptide transport protein A (gene amiA).
- Treponema pallidium 34 Kd antigen.
- Treponema pallidium membrane protein A (gene tmpA).
- Vibrio harveyi chitobiase (gene chb).
- Yersinia virulence plasmid protein yscJ.
- Halocyanin from Natrobacterium pharaonis [4], a membrane associated copper-binding protein. This is the first archaebacterial protein known to be modified in such a fashion).

From the precursor sequences of all these proteins, a consensus pattern and a set of rules to identify this type of post-translational modification were derived.

Consensus pattern: {DERK}(6)-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1)

The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence. Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT some 100 prokaryotic proteins. Some of them are not membrane lipoproteins, but at least half of them could be.

References

[1] Hayashi S., Wu H.C., J. Bioenerg. Biomembr. 22:451-471(1990).
[2] Klein P., Somorjai R.L., Lau P.C.K., Protein Eng. 2:15-20(1988).

[3] von Heijne G., Protein Eng. 2:531-534(1989).
[4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

856. (Lipoprotein_7)
Adhesin lipoprotein

This family consists of the p50 and variable adherence-associated antigen (Vaa) adhesins from Mycoplasma hominis. M. hominis is a mycoplasma associated with human urogenital diseases, pneumonia, and septic arthritis [1]. An adhesin is a cell surface molecule that mediates adhesion to other cells or to the surrounding surface or substrate. The Vaa antigen is a 50-kDa surface lipoprotein that has four tandem repetitive DNA sequences encoding a periodic peptide structure, and is highly immunogenic in the human host [1]. p50 is also a 50-kDa lipoprotein, having three repeats A,B and C, that may be a tetramer of 191-kDa in its native environment [2].

Number of members: 18

[1] Zhang Q, Wise KS; Medline: 96294788 “Molecular basis of size and antigenic variation of a Mycoplasma hominis adhesin encoded by divergent vaa genes. “ Infect Immun 1996;64:2737-2744.
[2] Henrich B, Kitzerow A, Feldmann RC, Schaal H, Hadding U; Medline: 97047675 “Repetitive elements of the Mycoplasma hominis adhesin p50 can be differentiated by monoclonal antibodies.” Infect Immun 1996;64:4027-4034.

857. (MaoC_like)
MaoC like domain

The MaoC protein is found to share similarity with a wide variety of enzymes; estradiol 17 beta-dehydrogenase 4, peroxisomal hydratase-dehydrogenase-epimerase, fatty acid synthase beta subunit. All these enzymes contain other domains. This domain is also present in the

NodN nodulation protein N. No specific function has been assigned to this region of any of these proteins. The maoC gene is part of a operon with maoA which is involved in the synthesis of monoamine oxidase [1].

Number of members: 46

[1] Sugino H, Sasaki M, Azakami H, Yamashita M, Murooka Y Medline: 96235221 “A monoamine-regulated Klebsiella aerogenes operon containing the monoamine oxidase structural gene (maoA) and the maoC gene.” J Bacteriol 1992;174:2485-2492.

858. (MSP)

Manganese-stabilizing protein / photosystem II polypeptide

This family consists of the 33 KDa photosystem II polypeptide from the oxygen evolving complex (OEC) of plants and cyanobacteria. The protein is also known as the manganese-stabilizing protein as it is associated with the manganese complex of the OEC and may provide the ligands for the complex [1].

Number of members: 17

[1] Philbrick JB, Zilinskas BA; Medline: 88334494 "Cloning, nucleotide sequence and mutational analysis of the gene encoding the Photosystem II manganese-stabilizing polypeptide of Synechocystis 6803.” Mol Gen Genet 1988;212:418-425.

859. (NAC)

[1] Makarova KS, Aravind L, Galperin MY, Grishin NV, Tatusov RL, Wolf YI, Koonin EV; Medline: 99342100 “Comparative genomics of the Archaea (Euryarchaeota): evolution of conserved protein families, the stable core, and the variable shell.” Genome Res 1999;9:608-628.

Number of members: 27

860. (Nop)

Putative snoRNA binding domain

This family consists of various Pre RNA processing ribonucleoproteins. The function of the aligned region is unknown however it may be a common RNA or snoRNA or Nop1p binding domain. Nop5p (Nop58p) Swiss:Q12499 from yeast is the protein component of a ribonucleoprotein protein required for pre-18s rRNA processing and is suggested to function with Nop1p in a snoRNA complex [1]. Nop56p Swiss:O00567 and Nop5p interact with Nop1p and are required for ribosome biogenesis [2]. Prp31p Swiss:p49704 is required for pre-mRNA splicing in S. cerevisiae [3].

Number of members: 23

[1] Wu P, Brockenbrough JS, Metcalfe AC, Chen S, Aris JP; Medline: 98298165 "Nop5p is a small nucleolar ribonucleoprotein component required for pre- 18 S rRNA processing in yeast." J Biol Chem 1998;273:16453-16463.
[2] Gautier T, Berges T, Tollervey D, Hurt E;Medline: 8038777 "Nucleolar KKE/D repeat proteins Nop56p and Nop58p interact with Nop1p and are required for ribosome biogenesis." Mol Cell Biol 1997;17:7088-7098.
[3] Weidenhammer EM, Singh M, Ruiz-Noriega M, Woolford JL Jr; Medline: 96184869 "The PRP31 gene encodes a novel protein required for pre-mRNA splicing in Saccharomyces cerevisiae." Nucleic Acids Res 1996;24:1164-1170.

861. (Nramp)

Natural resistance-associated macrophage protein

The natural resistance-associated macrophage protein (NRAMP) family consists of Nramp1, Nramp2, and yeast proteins Smf1 and Smf2. The NRAMP family is a novel family of functional related proteins defined by a conserved hydrophobic core of ten transmembrane domains [5]. This family of membrane proteins are divalent cation transporters. Nramp1 is an integral membrane protein expressed exclusively in cells of the immune system and is recruited to the membrane of a phagosome upon phagocytosis [1]. By controlling divalent cation concentrations Nramp1 may regulate the interphagosomal replication of bacteria [1]. Mutations in Nramp1 may genetically predispose an individual to susceptibility to diseases including leprosy and tuberculosis conversely this might however provide protection form rheumatoid arthritis [1]. Nramp2 is a multiple divalent cation transporter for Fe2+, Mn2+ and Zn2+ amongst others it is expressed at high levels in the intestine; and is major transferrin-independent iron uptake system in mammals [1]. The yeast proteins Smf1 and Smf2 may also transport divalent cations [3].

09595806.051400

Number of members: 36

[1] Govoni G, Gros P; Medline: 98383996 “Macrophage NRAMP1 and its role in resistance to microbial infections.” Inflamm Res 1998;47:277-284.

[2] Agranoff DD, Krishna S Medline: 98294035 “Metal ion homeostasis and intracellular parasitism.” Mol Microbiol 1998;28:403-412.

[3] Pinner E, Gruenheid S, Raymond M, Gros P; Medline: 98030569 “Functional complementation of the yeast divalent cation transporter family SMF by NRAMP2, a member of the mammalian natural resistance- associated macrophage protein family.” J Biol Chem 1997;272:28933-28938.

[4] Cellier M, Belouchi A, Gros P; Medline: 96402487 “Resistance to intracellular infections: comparative genomic analysis of Nramp.” Trends Genet 1996;12:201-204.

[5] Cellier M, Prive G, Belouchi A, Kwan T, Rodrigues V, Chia W, Gros P; Medline: 96036029 “Nramp defines a family of membrane proteins.” Proc Natl Acad Sci U S A 1995;92:10089-10093.

862. (NTP_transf_2)

Nucleotidyltransferase domain

Members of this family belong to a large family of nucleotidyltransferases [1].

Number of members: 83

[1] Holm L, Sander C; Medline: 96005605 “DNA polymerase beta belongs to an ancient nucleotidyltransferase superfamily.” Trends Biochem Sci 1995;20:345-347.

863. (Paramyxo_P)
Paramyxovirus P phosphoprotein

This family consists of paramyxovirus P phosphoprotein from sendai virus and human and bovine parainfluenza viruses. The P protein is an essential part of the viral RNA polymerase complex formed form the P and L proteins [1]. The exact role of the P protein in this complex in unknown but it is involved in multiple protein-protein interactions and binding the polymerase complex to the nucleocapsid or ribonucleoprotein template [1]. It also appears to be important for the proper folding of the L protein [1]. The paramyxoviruses have a negative sense ssRNA genome [1].

09695363.051502

Number of members: 15

[1] Bowman MC, Smallwood S, Moyer SA; Medline: 99329169 “Dissection of Individual Functions of the Sendai Virus Phosphoprotein in Transcription.” J Virol 1999;73:6474-6483.
[2] Matsuoka Y, Curran J, Pelet T, Kolakofsky D, Ray R, Compans RW; Medline: 91237868 “The P gene of human parainfluenza virus type 1 encodes P and C proteins but not a cysteine-rich V protein.” J Virol 1991;65:3406-3410.

864. (Patatin)

This family consists of various patatin glycoproteins from plants. The patatin protein accounts for up to 40% of the total soluble protein in potato tubers [2]. Patatin is a storage protein but it also has the enzymatic activity of lipid acyl hydrolase, catalysing the cleavage of fatty acids from membrane lipids [2].

Number of members: 21

[1] Banfalvi Z, Kostyal Z, Barta E; Medline: 95107249 “Solanum brevidens possesses a non-sucrose-inducible patatin gene.” Mol Gen Genet 1994;245:517-522.
[2] Mignery GA, Pikaard CS, Park WD; Medline: 88226014 “Molecular characterization of the patatin multigene family of potato.” Gene 1988;62:27-44.

865. (Pentapeptide_2)
Pentapeptide repeats (8 copies)

These repeats are found in many mycobacterial proteins. These repeats are most common in the PPE family of proteins, where they are found in the MPTR subfamily of PPE proteins. The function of these repeats is unknown. The repeat can be approximately described as XNXGX, where X can be any amino acid. These repeats are similar to Pentapeptide [1], however it is not clear if these two families are structurally related.

Number of members: 362

[1] Bateman A, Murzin A, Teichmann SA; Medline: 98318059 “Structure and distribution of pentapeptide repeats in bacteria.” Protein Sci 1998;7:1477-1480.
[2] Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Barrell BG; Medline: 98295987 “Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence.” Nature 1998;393:537-544.

866. (Peptidase_C13)
Peptidase C13 family

This family of peptidases is known as the hemoglobinase family because it contains a globin degrading enzyme from blood parasites Swiss:P42665. However relatives are found in plants and other organisms that have other functions. Members of this family are asparaginyl peptidases [1].

Number of members: 26

[1] Chen JM, Dando PM, Rawlings ND, Brown MA, Young NE, Stevens RA, Hewitt E, Watts C, Barrett AJ; Medline: 97218252 “Cloning, isolation, and characterization of mammalian legumain, an asparaginyl endopeptidase.” J Biol Chem 1997;272:8090-8098.

867. (Pro_dh)
Proline dehydrogenase

Number of members: 25

[1] Ling M, Allen SW, Wood JM; Medline: 95055736 “Sequence analysis identifies the proline dehydrogenase and delta 1- pyrroline-5-carboxylate dehydrogenase domains of the multifunctional Escherichia coli PutA protein.” J Mol Biol 1994;243:950-956.

868. (PsbP)

This family consists of the 23 kDa subunit of oxygen evolving system of photosystem II or PsbP from various plants (where it is encoded by the nuclear genome) and Cyanobacteria. The 23 KDa PsbP protein is required for PSII to be fully operational in vivo, it increases the affinity of the water oxidation site for Cl- and provides the conditions required for high affinity binding of Ca2+ [2].

Number of members: 25

[1] Rova EM, Mc Ewen B, Fredriksson PO, Styring S; Medline: 97067138 "Photoactivation and photoinhibition are competing in a mutant of Chlamydomonas reinhardtii lacking the 23-kDa extrinsic subunit of photosystem II." J Biol Chem 1996;271:28918-28924.

[2] Kochhar A, Khurana JP, Tyagi AK; Medline: 97191538 "Nucleotide sequence of the psbP gene encoding precursor of 23-kDa polypeptide of oxygen-evolving complex in Arabidopsis thaliana and its expression in the wild-type and a constitutively photomorphogenic mutant." DNA Res 1996;3:277-285.

869. (PUA)

The PUA domain named after PseudoUridine synthase and Archaeosine transglycosylase, was detected in archaeal and eukaryotic pseudouridine synthases, archaeal archaeosine synthases, a family of predicted ATPases that may be involved in RNA modification, a family of predicted archaeal and bacterial rRNA methylases. Additionally, the PUA domain was detected in a family of eukaryotic proteins that also contain a domain homologous to the translation initiation factor eIF1/SUI1; these proteins may comprise a novel type of translation factors. Unexpectedly, the PUA domain was detected also in bacterial and yeast glutamate kinases; this is compatible with the demonstrated role of these enzymes in the regulation of the expression of other genes [1]. It is predicted that the PUA domain is an RNA binding domain.

Number of members: 48

[1] Aravind L, Koonin EV; Medline: 99193178 "Novel predicted RNA-binding domains associated with the translation machinery." J Mol Evol 1999;48:291-302.

870. (RF1)

eRF1-like proteins

Members of this family are peptide chain release factors. The eukaryotic Release Factor 1 proteins (eRF1s) are involved in termination of translation. The eRF1 protein is functional for all stop codons and appears to abolish read-through of these codons. This family also includes other proteins for which the precise molecular function is unknown. Many of them are from Archaebacteria. These proteins may also be involved in translation termination but this awaits experimental verification. Number of members: 25

[1] Frolova L, Le Goff X, Rasmussen HH, Cheperegin S, Drugeon G, Kress M, Arman I, Haenni AL, Celis JE, Philippe M, et al; Medline: 95082951 “A highly conserved eukaryotic protein family possessing properties of polypeptide chain release factor” [see comments] Nature 1994;372:701-703.
[2] Drugeon G, Jean-Jean O, Frolova L, Le Goff X, Philippe M, Kisselev L, Haenni AL; Medline: 97315314 “Eukaryotic release factor 1 (eRF1) abolishes readthrough and competes with suppressor tRNAs at all three termination codons in messenger RNA.” Nucleic Acids Res 1997;25:2254-2258.

871. (Ribosomal_L14e)Ribosomal protein L14
This family includes the eukaryotic ribosomal protein L14.
Number of members: 15

872. (Ribosomal_S27)
Ribosomal protein S27a

This family of ribosomal proteins consists mainly of the 40S ribosomal protein S27a which is synthesized as a C-terminal extension of ubiquitin (CEP). The S27a domain compromises the C-terminal half of the protein. The synthesis of ribosomal proteins as extensions of ubiquitin promotes their incorporation into nascent ribosomes by a transient metabolic stabilization and is required for efficient ribosome biogenesis [3]. The ribosomal extension protein S27a contains a basic region that is proposed to form a zinc finger; its fusion gene is proposed as a mechanism to maintain a fixed ratio between ubiquitin necessary for degrading proteins and ribosomes a source of proteins [2].

Number of members: 36

873. (Spermine_synth)

Spermine/spermidine synthase

Spermine and spermidine are polyamines. This family includes spermidine synthase that catalyses the fifth (last) step in the biosynthesis of spermidine from arginine, and spermine synthase.

Number of members: 39

[1] Mezquita J, Pau M, Mezquita C; Medline: 97449308 “Characterization and expression of two chicken cDNAs encoding ubiquitin fused to ribosomal proteins of 52 and 80 amino acids.” Gene 1997;195:313-319.
[2] Redman KL, Rechsteiner M; Medline: 89181932 “Identification of the long ubiquitin extension as ribosomal protein S27a.” Nature 1989;338:438-440.
[3] Finley D, Bartel B, Varshavsky A; Medline: 89181925 “The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis.” Nature 1989;338:394-401.

874. (Surp)

Surp module

[1] Denhez F, Lafyatis R; Medline: 94266805 “Conservation of regulated alternative splicing and identification of functional domains in vertebrate homologs to the Drosophila splicing regulator, suppressor-of-white-apricot.” J Biol Chem 1994;269:16170-16179.

This domain is also known as the SWAP domain. SWAP stands for Suppressor-of-White-APricot. It has been suggested that these domains may be RNA binding [1].

Number of members: 32

875. (TFIIE)

TFIIE alpha subunit

The general transcription factor TFIIE has an essential role in eukaryotic transcription initiation together with RNA polymerase II and other general factors. Human TFIIE consists of two subunits TFIIE-alpha Swiss:P29083 and TFIIE-beta Swiss:P29084 and joins the preinitiation complex after RNA polymerase II and TFIIF [1]. This family consists of the conserved amino terminal region of eukaryotic TFIIE-alpha [2] and proteins from archaebacteria that are presumed to be TFIIE-alpha subunits also Swiss:O29501 [3].

Number of members: 12

[1] Ohkuma Y, Sumimoto H, Hoffmann A, Shimasaki S, Horikoshi M, Roeder RG; Medline: 92065982 “Structural motifs and potential sigma homologies in the large subunit of human general transcription factor TFIIE.” Nature 1991;354:398-401.
[2] Ohkuma Y, Hashimoto S, Roeder RG, Horikoshi M; Medline: 93087200 Identification of two large subdomains in TFIIE-alpha on the basis of homology between Xenopus and human sequences. Nucleic Acids Res 1992;20:5838-5838.
[3] Klenk HP, Clayton RA, Tomb JF, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, Richardson DL, Kerlavage AR, Graham DE, Kyrpides NC, Fleischmann RD, Quackenbush J, Lee NH, Sutton GG, Gill S, Kirkness EF, Dougherty BA, McKenney K, Adams MD, Loftus B, Venter JC, et al; Medline: 98049343 “The complete genome sequence of the hyperthermophilic, sulphate- reducing archaeon Archaeoglobus fulgidus.” Nature 1997;390:364-370.

876. (Transglut_core)

Cross-reference(s) PS00547; TRANSGLUTAMINASES

Transglutaminases (EC 2.3.2.13) (TGase) [1,2] are calcium-dependent enzymes that catalyze the cross-linking of proteins by promoting the formation of isopeptide bonds between the gamma-carboxyl group of a glutamine in one polypeptide chain and the epsilon-amino group of a lysine in a second polypeptide chain. TGases also catalyze the conjugation of polyamines to proteins. The best known transglutaminase is blood coagulation factor XIII, a plasma tetrameric protein composed of two catalytic A subunits and two non-catalytic B subunits. Factor XIII is responsible for cross-linking fibrin chains, thus stabilizing the fibrin clot. Other forms of transglutaminases are widely distributed in various organs, tissues and body fluids. Sequence data is available for the following forms of TGase:

- Transglutaminase K (Tgase K), a membrane-bound enzyme found in mammalian epidermis and important for the formation of the cornified cell envelope (gene TGM1).
- Tissue transglutaminase (TGase C), a monomeric ubiquitous enzyme located in the cytoplasm (gene TGM2).
- Transglutaminase 3, responsible for the later stages of cell envelope formation in the epidermis and the hair follicle (gene TGM3).
- Transglutaminase 4 (gene TGM4).

A conserved cysteine is known to be involved in the catalytic mechanism of TGases. The erythrocyte membrane band 4.2 protein, which probably plays an important role in regulating the shape of erythrocytes and their mechanical properties, is evolutionary related to TGases. However the active site cysteine is substituted by an alanine and the 4.2 protein does not show TGase activity.

Consensus pattern:[GT]-Q-[CA]-W-V-x-[SA]-[GA]-[IVT]-x(2)-T-x-[LMSC]-R-[CSA]-[LV]-G [The first C is the active site residue]   Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

[1] Ichinose A., Bottenus R.E., Davie E.W. J. Biol. Chem. 265:13411-13414(1990).
[2] Greenberg C.S., Birckbichler P.J., Rice R.H. FASEB J. 5:3071-3077(1991).

877. (TruB_N)

TruB family pseudouridylate synthase (N terminal domain)

Members of this family are involved in modifying bases in RNA molecules. They carry out the conversion of uracil bases to pseudouridine. This family includes TruB, a pseudouridylate synthase that specifically converts uracil 55 to pseudouridine in most tRNAs. This family also includes Cbf5p that modifies rRNA [2].

Number of members: 33

[1] Nurse K, Wrzesinski J, Bakin A, Lane BG, Ofengand J; Medline: 96079944 "Purification, cloning, and properties of the tRNA psi 55 synthase from Escherichia coli." RNA 1995;1:102-112.

[2] Lafontaine DLJ, Bousquet-Antonelli C, Henry Y, Caizergues-Ferrer M, Tollervey D; Medline: 98139521 "The box H + ACA snoRNAs carry Cbf5p, the putative rRNA pseudouridine synthase." Genes Dev 1998;12:527-537.

09565359.051601

878. (UDPGP)

UTP--glucose-1-phosphate uridylyltransferase

This family consists of UTP--glucose-1-phosphate uridylyltransferases, EC:2.7.7.9. Also known as UDP-glucose pyrophosphorylase (UDPGP) and Glucose-1-phosphate uridylyltransferase. UTP--glucose-1-phosphate uridylyltransferase catalyses the interconversion of MgUTP + glucose-1-phosphate and UDP-glucose + MgPPi [1]. UDP-glucose is an important intermediate in mammalian carbohydrate interconversion involved in various metabolic roles depending on tissue type [1]. In Dictyostelium (slime mold) mutants in this enzyme abort the development cycle [2]. Also within the family is UDP-N-acetylglucosamine Swiss:Q16222 or AGX1 [3] and two hypothetical proteins from Borrelia burgdorferi the lyme disease spirochaete Swiss:O51893 and Swiss:O51036.

Number of members: 18

[1] Duggleby RG, Chao YC, Huang JG, Peng HL, Chang HY; Medline: 96202932 "Sequence differences between human muscle and liver cDNAs for UDPglucose pyrophosphorylase and kinetic properties of the recombinant enzymes expressed in Escherichia coli." Eur J Biochem 1996;235:173-179.

[2] Ragheb JA, Dottin RP; Medline: 87231075 "Structure and sequence of a UDP glucose pyrophosphorylase gene of Dictyostelium discoideum." Nucleic Acids Res 1987;15:3891-3906.

[3] Mio T, Yabe T, Arisawa M, Yamada-Okabe H; Medline: 98269105 "The eukaryotic UDP-N-acetylglucosamine pyrophosphorylases. Gene cloning, protein expression, and catalytic mechanism. J Biol Chem 1998;273:14392-14397.

09553250.071000

879. (UPF004)

Uncharacterized protein family UPF0044 signature

Cross-reference(s) PS01301; UPF0044

The following uncharacterized proteins have been shown [1] to be highlysimilar:

- Bacillus subtilis hypothetical protein yqeI.
- Escherichia coli hypothetical protein yhbY and HI1333, the corresponding Haemophilus influenzae protein.
- Methanococcus jannaschii hypothetical protein MJ0652.

These are small proteins of 10 to 15 Kd. They can be picked up in the database by the following pattern. This pattern is located in the N-terminal part of these proteins.

Consensus pattern: L-[ST]-x(3)-K-x(3)-[KR]-[SGA]-x-[GA]-H-x-L-x-P-[LIV]-x(2)- [LIV]-[GA]-x(2)-G Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

880. (zf-A20)

A20-like zinc finger

A20- (an inhibitor of cell death)-like zinc fingers. The zinc finger mediates self-association in A20. These fingers also mediate IL-1-induced NF-kappa B activation.

Number of members: 22

[1] Heyninck K, Beyaert R; Medline: 99126071 "The cytokine-inducible zinc finger protein A20 inhibits IL-1-induced NF- kappaB activation at the level of TRAF6. FEBS Lett 1999;442:147-150.

[2] De Valck D, Heyninck K, Van Criekinge W, Contreras R,Beyaert R, Fiers W; Medline: 96390831 "A20, an inhibitor of cell death, self-associates by its zinc finger domain." FEBS Lett 1996;384:61-64.

[3] Song HY, Rothe M, Goeddel DV; Medline: 96270609 "The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-kappaB activation. Proc Natl Acad Sci U S A 1996;93:6721-6725.

[4] Opipari AW Jr, Boguski MS, Dixit VM; Medline: 90368626 "The A20 cDNA induced by tumor necrosis factor alpha encodes a novel type of zinc finger protein." J Biol Chem 1990;265:14705-14708.

09955636.070601

881. (zf-PARP)

Poly(ADP-ribose) polymerase zinc finger domain

Cross-reference(s) PS00347; PARP_ZN_FINGER_1 PS50064; PARP_ZN_FINGER_2

Poly(ADP-ribose) polymerase (EC 2.4.2.30) (PARP) [1,2] is a eukaryotic enzyme that catalyzes the covalent attachment of ADP-ribose units from NAD(+) to various nuclear acceptor proteins. This post-translational modification of nuclear proteins is dependent on DNA. It appears to be involved in the regulation of various important cellular processes such as differentiation, proliferation and tumor transformation as well as in the regulation of the molecular events involved in the recovery of the cell from DNA damage. Structurally, PARP, about 1000 amino-acids residues long, consists of three distinct domains: an N-terminal zinc-dependent DNA-binding domain, a central automodification domain and a C-terminal NAD-binding domain. The DNA-binding region contains a pair of zinc finger domains which have been shown to bind DNA in a zinc-dependent manner. The zinc finger domains of PARP seem to bind specifically to single-stranded DNA. DNA ligase III [3] contains, in its N-terminal section, a single copy of a zinc finger highly similar to those of PARP.

Consensus pattern: C-[KR]-x-C-x(3)-I-x-K-x(3)-[RG]-x(16,18)-W-[FYH]-H-x(2)-C [The three C's and the H are zinc ligands] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE. Sequences known to belong to this class detected by the profile ALL. Other sequence(s) detected in SWISS-PROTNONE.

Note: This documentation entry is linked to both signature patterns and a profile. As the profile is much more sensitive than the patterns, you should use it if you have access to the necessary software tools to do so.

09855362.071502

[1] Althaus F.R., Richter C.R. Mol. Biol. Biochem. Biophys. 37:1-126(1987).
[2] de Murcia G., Menissier de Murcia J. Trends Biochem. Sci. 19:172-176(1994).
[3] Wei Y.-F., Robins P., Carter K., Caldecott K., Pappin D.J.C., Yu G.-L., Wang R.-P., Shell B.K., Nash R.A., Schar P., Barnes D.E., Haseltine W.A., Lindahl T. Mol. Cell. Biol. 15:3206-3216(1995).

882. Adenylylsulfate kinase (APS_kinase)
Enzyme that catalyses the phosphorylation of adenylylsulfate to 3'-phosphoadenylylsulfate. This domain contains an ATP binding P-loop motif. Number of members: 34

[1] MacRae IJ, Rose AB, Segel IH; Medline: 99003196 “Adenosine 5'-phosphosulfate kinase from Penicillium chrysogenum. site- directed mutagenesis at putative phosphoryl-accepting and ATP P-loop residues. J Biol Chem 1998;273:28583-28589.

883. DNA polymerase family B signature DNA_POLYMERASE_B (DNA_pol_B)

Replicative DNA polymerases (EC 2.7.7.7) are the key enzymes catalyzing the accurate replication of DNA. They require either a small RNA molecule or a protein as a primer for the de novo synthesis of a DNA chain. On the basis of sequence similarity, a number of DNA polymerases have been grouped [1 to 7] under the designation of DNA polymerase family B. These are:

- Higher eukaryotes polymerases alpha.
- Higher eukaryotes polymerases delta.
- Yeast polymerase I/alpha (gene POL1), polymerase II/epsilon (gene POL2), polymerase III/delta (gene POL3) and polymerase REV3.
- Escherichia coli polymerase II (gene dinA or polB).
- Archaebacterial polymerases.
- Polymerases of viruses from the herpesviridae family.
- Polymerases from Adenoviruses.
- Polymerases from Baculoviruses.
- Polymerases from Chlorella viruses.
- Polymerases from Poxviruses.
- Bacteriophage T4 polymerase.
- Podoviridae bacteriophages Phi-29, M2 and PZA polymerase.
- Tectiviridae bacteriophage PRD1 polymerase.
- Polymerases encoded on mitochondrial linear DNA plasmids in various fungi and plants (Kluyveromyces lactis pGKL1 and pGKL2, Agaricus bitorquis pEM, Ascobolus immersus pAI2, Claviceps purpurea pCLK1, Neurospora Kalilo and Maranhar, maize S-1, etc).

Six regions of similarity (numbered from I to VI) are found in all or a subset of the above polymerases. The most conserved region (I) includes a conserved tetrapeptide with two aspartate residues. Its function is not yet known. However, it has been suggested [3] that it may be involved in binding a magnesium ion. This conserved region was selected as a signature for this family of DNA polymerases.

Consensus pattern [YA]-[GLIVMSTAC]-D-T-D-[SG]-[LIVMFTC]-x-[LIVMSTAC]
Sequences known to belong to this class detected by the patternALL, except for yeast polymerase II/epsilon, Agaricus bitorquis pEM and Sulfolobus solfataricus polymerase II.

[1] Jung G., Leavitt M.C., Hsieh J.-C., Ito J. Proc. Natl. Acad. Sci. U.S.A. 84:8287-8291(1987).

[2] Bernad A., Zaballos A., Salas M., Blanco L. EMBO J. 6:4219-4225(1987).

[3] Argos P. Nucleic Acids Res. 16:9909-9916(1988).

[4] Wang T.S.-F., Wong S.W., Korn D. FASEB J. 3:14-21(1989).

[5] Delarue M., Poch O., Todro N., Moras D., Argos P. Protein Eng. 3:461-467(1990).

[6] Ito J., Braithwaite D.K. Nucleic Acids Res. 19:4045-4057(1991).

[7] Braithwaite D.K., Ito J. Nucleic Acids Res. 21:787-802(1993).

884. DNA polymerase family X signature - DNA_POLYMERASE_X (DNA_polymeraseX)

DNA polymerases (EC 2.7.7.7) can be classified, on the basis of sequence similarity [1], into at least four different groups: A, B, C and X. DNA polymerases that belong to family X are listed below [2]:

- Vertebrate polymerase beta, involved in DNA repair.
- Yeast polymerase IV (POL4) [3], an enzyme with similar characteristics to that of the mammalian polymerase beta.
- Terminal deoxynucleotidyltransferase (TdT) (EC 2.7.7.31). TdT catalyzes the elongation of polydeoxynucleotide chains by terminal addition. One of the functions of this enzyme is the addition of nucleotides at the junction of rearranged Ig heavy chain and T cell receptor gene segments during the maturation of B and T cells.
- African Swine Fever virus protein O174L [4].
- Fission yeast hypothetical protein SpAC2F7.06c.

These enzymes are small (about 40 Kd) compared with other polymerases and their reaction mechanism operates via a distributive mode, i.e. they dissociate from the template-primer after addition of each nucleotide.

As a signature pattern for this family of DNA polymerases, a highly conserved region that contains a conserved arginine and two conserved aspartic acid residues were selected. The latter together with the arginine have been shown [5] to be involved in primer binding in polymerase beta.

Consensus pattern G-[SG]-[LFY]-x-R-[GE]-x(3)-[SGCL]-x-D-[LIVM]-D- [LIVMFY](3)-x(2)-[SAP] Sequences known to belong to this class detected by the patternALL.

[1] Ito J., Braithwaite D.K. Nucleic Acids Res. 19:4045-4057(1991).

[2] Matsukage A., Nishikawa K., Ooi T., Seto Y., Yamaguchi M. J. Biol. Chem. 262:8960-8962(1987).

[3] Prasad R., Widen S.G., Singhal R.K., Watkins J., Prakash L., Wilson S.H. Nucleic Acids Res. 21:5301-5307(1993).

[4] Yanez R.J., Rodriguez J.M., Nogal M.L., Yuste L., Enriquez C., Rodriguez J.F., Vinuela E. Virology 208:249-278(1995).

[5] Date T., Yamamoto S., Tanihara K., Nishimoto Y., Matsukage A. Biochemistry 30:5286-5292(1991).

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885. DUF14 - Domain of unknown function

This domain is found in glutamate synthase, tungsten formylmethanofuran dehydrogenase subunit c (FwdC) and molybdenum formylmethanofuran dehydrogenase subunit c (FmdC). It has no known function. Number of members: 52

[1] Hochheimer A, Hedderich R, Thauer RK; Medline: 99035764. “The formylmethanofuran dehydrogenase isoenzymes in Methanobacterium wolfei and Methanobacterium thermoautotrophicum: induction of the molybdenum isoenzyme by molybdate and constitutive synthesis of the tungsten isoenzyme.” Arch Microbiol 1998;170:389-393.

886. DUF18-Domain of unknown function

This domain of unknown function is found in several C. elegans proteins. The domain is 120 amino acids long and rich in cysteine residues. There are 16 conserved cysteine positions in the domain. Number of members: 34

887. DUF27-Domain of unknown function

This domain is found in a number of otherwise unrelated proteins. This domain is found at the C-terminus of the macro-H2A histone protein Swiss:Q02874. This domain is found in the non-structural proteins of several types of ssRNA viruses such as NSP2 from alphaviruses Swiss:P03317. This domain is also found on its own in a family of proteins from bacteria

Swiss:P75918, archaebacteria Swiss:O59182 and eukaryotes Swiss:Q17432, suggesting that it is involved in an important and ubiquitous cellular process. Number of members: 66

888. DUF37-Domain of unknown function

This domain is found in short (70 amino acid) hypothetical proteins from various bacteria. The domain contains three conserved cysteine residues. Swiss:Q44066 from Aeromonas hydrophila has been found to have hemolytic activity (unpublished). Number of members: 19

889. EGF-like domain signatures. (EGF-like)

A sequence of about thirty to forty amino-acid residues long found in the sequence of epidermal growth factor (EGF) has been shown [1 to 6] to be present, in a more or less conserved form, in a large number of other, mostly animal proteins. The proteins currently known to contain one or more copies of an EGF-like pattern are listed below.

- Adipocyte differentiation inhibitor (gene PREF-1) from mouse (6 copies).
- Agrin, a basal lamina protein that causes the aggregation of acetylcholine receptors on cultured muscle fibers (4 copies).
- Amphiregulin, a growth factor (1 copy).
- Betacellulin, a growth factor (1 copy).
- Blastula proteins BP10 and Span from sea urchin which are thought to be involved in pattern formation (1 copy).
- BM86, a glycoprotein antigen of cattle tick (7 copies).
- Bone morphogenic protein 1 (BMP-1), a protein which induces cartilage and bone formation and which expresses metalloendopeptidase activity (1-2 copies). Homologous proteins are found in sea urchin - suBMP (1 copy) - and in Drosophila - the dorsal-ventral patterning protein tolloid (2 copies).
- Caenorhabditis elegans developmental proteins lin-12 (13 copies) and glp-1 (10 copies).
- Caenorhabditis elegans APX-1 protein, a patterning protein (4.5 copies).
- Calcium-dependent serine proteinase (CASP) which degrades the extracellular matrix proteins type I and IV collagen and fibronectin (1 copy).
- Cartilage matrix protein CMP (1 copy).
- Cartilage oligomeric matrix protein COMP (4 copies).
- Cell surface antigen 114/A10 (3 copies).

- Cell surface glycoprotein complex transmembrane subunit ASGP-2 from rat (2 copies).
- Coagulation associated proteins C, Z (2 copies) and S (4 copies).
- Coagulation factors VII, IX, X and XII (2 copies).
- Complement C1r components (1 copy).
- Complement C1s components (1 copy).
- Complement-activating component of Ra-reactive factor (RARF) (1 copy).
- Complement components C6, C7, C8 alpha and beta chains, and C9 (1 copy).
- Crumbs, an epithelial development protein from Drosophila (29 copies).
- Epidermal growth factor precursor (7-9 copies).
- Exogastrula-inducing peptides A, C, D and X from sea urchin (1 copy).
- Fat protein, a Drosophila cadherin-related tumor suppressor (5 copies).
- Fetal antigen 1, a probable neuroendocrine differentiation protein, which is derived from the delta-like protein (DLK) (6 copies).
- Fibrillin 1 (47 copies) and fibrillin 2 (14 copies).
- Fibropellins IA (21 copies), IB (13 copies), IC (8 copies), II (4 copies) and III (8 copies) from the apical lamina - a component of the extracellular matrix - of sea urchin.
- Fibulin-1 and -2, two extracellular matrix proteins (9-11 copies).
- Giant-lens protein (protein Argos), which regulates cell determination and axon guidance in the Drosophila eye (1 copy).
- Growth factor-related proteins from various poxviruses (1 copy).
- Gurken protein, a Drosophila developmental protein (1 copy).
- Heparin-binding EGF-like growth factor (HB-EGF), transforming growth factor alpha (TGF-alpha), growth factors Lin-3 and Spitz (1 copy); the precursors are membrane proteins, the mature form is located extracellular.
- Hepatocyte growth factor (HGF) activator (EC 3.4.21.-) (2 copies).
- LDL and VLDL receptors, which bind and transport low-density lipoproteins and very low-density lipoproteins (3 copies).
- LDL receptor-related protein (LRP), which may act as a receptor for endocytosis of extracellular ligands (22 copies).
- Leucocyte antigen CD97 (3 copies), cell surface glycoprotein EMR1 (6 copies) and cell surface glycoprotein F4/80 (7 copies).
- Limulus clotting factor C, which is involved in hemostasis and host defense mechanisms in japanese horseshoe crab (1 copy).

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- Meprin A alpha subunit, a mammalian membrane-bound endopeptidase (1 copy).
- Milk fat globule-EGF factor 8 (MFG-E8) from mouse (2 copies).
- Neuregulin GGF-I and GGF-II, two human glial growth factors (1 copy).
- Neurexins from mammals (3 copies).
- Neurogenic proteins Notch, Xotch and the human homolog Tan-1 (36 copies), Delta (9 copies) and the similar differentiation proteins Lag-2 from Caenorhabditis elegans (2 copies), Serrate (14 copies) and Slit (7 copies) from Drosophila.
- Nidogen (also called entactin), a basement membrane protein from chordates (2-6 copies).
- Ookinete surface proteins (24 Kd, 25 Kd, 28 Kd) from Plasmodium (4 copies).
- Pancreatic secretory granule membrane major glycoprotein GP2 (1 copy).
- Perforin, which lyses non-specifically a variety of target cells (1 copy).
- Proteoglycans aggrecan (1 copy), versican (2 copies), perlecan (at least 2 copies), brevican (1 copy) and chondroitin sulfate proteoglycan (gene PG-M) (2 copies).
- Prostaglandin G/H synthase 1 and 2 (EC 1.14.99.1) (1 copy), which is found in the endoplasmatic reticulum.
- S1-5, a human extracellular protein whose ultimate activity is probably modulated by the environment (5 copies).
- Schwannoma-derived growth factor (SDGF), an autocrine growth factor as well as a mitogen for different target cells (1 copy).
- Selectins. Cell adhesion proteins such as ELAM-1 (E-selectin), GMP-140 (P-selectin), or the lymph-node homing receptor (L-selectin) (1 copy).
- Serine/threonine-protein kinase homolog (gene Pro25) from Arabidopsis thaliana, which may be involved in assembly or regulation of light-harvesting chlorophyll A/B protein (2 copies).
- Sperm-egg fusion proteins PH-30 alpha and beta from guinea pig (1 copy).
- Stromal cell derived protein-1 (SCP-1) from mouse (6 copies).
- TDGF-1, human teratocarcinoma-derived growth factor 1 (1 copy).
- Tenascin (or neuronectin), an extracellular matrix protein from mammals (14.5 copies), chicken (TEN-A) (13.5 copies) and the related proteins human tenascin-X (18 copies) and tenascin-like proteins TEN-A and TEN-M from Drosophila (8 copies).
- Thrombomodulin (fetomodulin), which together with thrombin activates protein C (6 copies).

- Thrombospondin 1, 2 (3 copies), 3 and 4 (4 copies), adhesive glycoproteins that mediate cell-to-cell and cell-to-matrix interactions.
- Thyroid peroxidase 1 and 2 (EC 1.11.1.8) from human (1 copy).
- Transforming growth factor beta-1 binding protein (TGF-B1-BP) (16 or 18 copies).
- Tyrosine-protein kinase receptors Tek and Tie (EC 2.7.1.112) (3 copies).
- Urokinase-type plasminogen activator (EC 3.4.21.73) (UPA) and tissue plasminogen activator (EC 3.4.21.68) (TPA) (1 copy).
- Uromodulin (Tamm-horsfall urinary glycoprotein) (THP) (3 copies).
- Vitamin K-dependent anticoagulants protein C (2 copies) and protein S (4 copies) and the similar protein Z, a single-chain plasma glycoprotein of unknown function (2 copies).
- 63 Kd sperm flagellar membrane protein from sea urchin (3 copies).
- 93 Kd protein (gene nel) from chicken (5 copies).
- Hypothetical 337.6 Kd protein T20G5.3 from Caenorhabditis elegans (44 copies).

The functional significance of EGF domains in what appear to be unrelated proteins is not yet clear. However, a common feature is that these repeats are found in the extracellular domain of membrane-bound proteins or in proteins known to be secreted (exception: prostaglandin G/H synthase). The EGF domain includes six cysteine residues which have been shown (in EGF) to be involved in disulfide bonds. The main structure is a two-stranded beta-sheet followed by a loop to a C-terminal short two-stranded sheet. Subdomains between the conserved cysteines strongly vary in length as shown in the following schematic representation of the EGF-like domain:

```
              +-------------------+       +-------------------------+            |            |     |
 | x(4)-C-x(0,48)-C-x(3,12)-C-x(1,70)-C-x(1,6)-C-x(2)-G-a-x(0,21)-G-x(2)-C-x      |
 |        ************************************
        +-------------------+
```

'C': conserved cysteine involved in a disulfide bond.
'G': often conserved glycine
'a': often conserved aromatic amino acid
'*': position of both patterns.
'x': any residue

The region between the 5th and 6th cysteine contains two conserved glycines of which at least one is present in most EGF-like domains. Two patterns were created for this domain, each including one of these C-terminal conserved glycine residues.

Consensus pattern: C-x-C-x(5)-G-x(2)-C [The 3 C's are involved in disulfide bonds] Sequences known to belong to this class detected by the pattern A majority, but not those that have very long or very short regions between the last 3 conserved cysteines of their EGF-like domain(s). Other sequence(s) detected in SWISS-PROT87 proteins, of which 27 can be considered as possible candidates.

Consensus pattern: C-x-C-x(2)-[GP]-[FYW]-x(4,8)-C [The three C's are involved in disulfide bonds]Sequences known to belong to this class detected by the patternA majority, but not those that have very long or very short regions between the last 3 conserved cysteines of their EGF-like domain(s). Other sequence(s) detected in SWISS-PROT83 proteins, of which 49 can be considered as possible candidates. Note The beta chain of the integrin family of proteins contains 2 cysteine- rich repeats which were said to be dissimilar with the EGF pattern [7].

Note Laminin EGF-like repeats (see <PDOC00961>) are longer than the average EGF module and contain a further disulfide bond C-terminal of the EGF-like region. Perlecan and agrin contain both EGF-like domains and laminin-type EGF-like domains. Note the pattern do not detect all of the repeats of proteins with multiple EGF-like repeats. Note see <PDOC00913> for an entry describing specifically the subset of EGF- like domains that bind calcium.

[1] Davis C.G. New Biol. 2:410-419(1990).
[2] Blomquist M.C., Hunt L.T., Barker W.C. Proc. Natl. Acad. Sci. U.S.A. 81:7363-7367(1984).
[3] Barker W.C., Johnson G.C., Hunt L.T., George D.G. Protein Nucl. Acid Enz. 29:54-68(1986).
[4] Doolittle R.F., Feng D.F., Johnson M.S. Nature 307:558-560(1984).
[5] Appella E., Weber I.T., Blasi F. FEBS Lett. 231:1-4(1988).
[6] Campbell I.D., Bork P. Curr. Opin. Struct. Biol. 3:385-392(1993).

[7] Tamkun J.W., DeSimone D.W., Fonda D., Patel R.S., Buck C., Horwitz A.F., Hynes R.O. Cell 46:271-282(1986).

890. Ham1 family (Ham1p_like)

This family consists of the HAM1 protein Swiss:P47119 and hypothetical archaeal bacterial and C. elegans proteins. HAM1 controls 6-N-hydroxylaminopurine (HAP) sensitivity and mutagenesis in S. cerevisiae Swiss:P47119 [1]. The HAM1 protein protects the cell from HAP, either on the level of deoxynucleoside triphosphate or the DNA level by a yet unidentified set of reactions [1]. Number of members: 19

[1] Noskov VN, Staak K, Shcherbakova PV, Kozmin SG, Negishi K, Ono BC, Hayatsu H, Pavlov YI; Medline: 96381244 “HAM1, the gene controlling 6-N-hydroxylaminopurine sensitivity and mutagenesis in the yeast Saccharomyces cerevisiae.” Yeast 1996;12:17-29.

891. (HCO3_cotransp)

Anion exchange is a cellular transport function which contributes to the regulation of cell pH and volume. Anion exchangers are a family of functionally related proteins that contributes to these properties by maintaining the intracellular level of the two principal anions: chloride and HCO3-. The best characterized anion exchanger is the band 3 protein [1], which is an erythrocyte anion exchange membrane glycoprotein. Band 3 is a protein of about 900 amino acids which consists of a cytoplasmic N-terminal domain of about 400 residues and an hydrophobic C-terminal section of about 500 residues that contains at least ten transmembrane regions. The cytoplasmic domain provides binding sites for cytoskeletal proteins, while the integral membrane domain is responsible for anion transport. Band 3 protein is specific to erythroid cells, at least two other proteins [2] structurally and functionally related to band 3, are found in nonerythroid tissues:

- AE2 (or B3 related protein; B3RP), a protein of 1200 residues, which seems to be present in a variety of cell types including lymphoid, kidney, and choroid plexus.
- AE3, a protein of 1200 residues, which is specific to neurons.

Structurally AE2 and AE3 are very similar to band 3, the main difference being an extension of some 300 residues of the N-terminal domain in AE2 and AE3.

Two signature patterns were developed for these proteins. The first pattern is based on a conserved stretch of sequence that contains four clustered positive charged residues and which is located at the C-terminal extremity of the cytoplasmic domain, just before the first transmembrane segment from the integral domain. The second pattern is based on the perfectly conserved sequence of the fifth transmembrane segment; this segment contains a lysine, which is the covalent binding site for the isothiocyanate group of DIDS, an inhibitor of anion exchange.

Consensus pattern F-G-G-[LIVM](2)-[KR]-D-[LIVM]-[RK]-R-R-Y Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [FI]-L-I-S-L-I-F-I-Y-E-T-F-x-K-L Sequences known to belong to this class detected by the pattern ALL.

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[1] Jay D., Cantley L. Annu. Rev. Biochem. 55:511-538(1986).
[2] Reithmeier R.A.F. Curr. Opin. Struct. Biol. 3:515-523(1993).

892. ATP phosphoribosyltransferase signature (HisG)
ATP phosphoribosyltransferase (EC 2.4.2.17) is the enzyme that catalyzes the first step in the biosynthesis of histidine in bacteria, fungi and plants. It is a protein of about 23 to 32 Kd. As a signature pattern a region located in the C-terminal part of this enzyme was selected.

Consensus pattern E-x(5)-G-x-[SAG]-x(2)-[IV]-x-D-[LIV]-x(2)-[ST]-G-x-T-[LM] Sequences known to belong to this class detected by the pattern ALL.

893. HNH endonuclease (HNH)
Number of members: 56
[1] Shub DA, Goodrich-Blair H, Eddy SR; Medline: 95117127 “Amino acid sequence motif of group I intron endonucleases is conserved in open reading frames of group II introns.” Trends Biochem Sci 1994;19:402-404.

[2] Dalgaard JZ, Klar AJ, Moser MJ, Holley WR, Chatterjee A, Mian IS; Medline: 98026854 "Statistical modeling and analysis of the LAGLIDADG family of site- specific endonucleases and identification of an intein that encodes a site-specific endonuclease of the HNH family." Nucleic Acids Res 1997;25:4626-4638.

[3] Gorbalenya AE; Medline: 95004046 "Self-splicing group I and group II introns encode homologous (putative) DNA endonucleases of a new family." Protein Sci 1994;3:1117-1120.

894. NEUROHYPOPHYS_HORM (hormone5)

Oxytocin (or ocytocin) and vasopressin [1] are small (nine amino acid residues), structurally and functionally related neurohypophysial peptide hormones. Oxytocin causes contraction of the smooth muscle of the uterus and of the mammary gland while vasopressin has a direct antidiuretic action on the kidney and also causes vasoconstriction of the peripheral vessels. Like the majority of active peptides, both hormones are synthesized as larger protein precursors that are enzymatically converted to their mature forms. Peptides belonging to this family are also found in birds, fish, reptiles and amphibians (mesotocin, isotocin, valitocin, glumitocin, aspargtocin, vasotocin, seritocin, asvatocin, phasvatocin), in worms (annetocin), octopi (cephalotocin), locust (locupressin or neuropeptide F1/F2) and in molluscs (conopressins G and S) [2]. The pattern developed to detect this category of peptides spans their entire sequence and includes four invariant amino acid residues.

Consensus pattern C-[LIFY](2)-x-N-[CS]-P-x-G [The two C's are linked by a disulfide bond]. Sequences known to belong to this class detected by the pattern ALL.

[1] Acher R., Chauvet J. Biochimie 70:1197-1207(1988).

[2] Chauvet J., Michel G., Ouedraogo Y., Chou J., Chait B.T., Acher R. Int. J. Pept. Protein Res. 45:482-487(1995).

895. 7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (HPPK)

All organisms require reduced folate cofactors for the synthesis of a variety of metabolites. Most microorganisms must synthesize folate de novo because they lack the active transport system of higher vertebrate cells which allows these organisms to use dietary folates. Enzymes involved in folate biosynthesis are therefore targets for a variety of antimicrobial agents such as trimethoprim or sulfonamides. 7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (EC 2.7.6.3) (HPPK) catalyzes the attachment of pyrophosphate to 6-hydroxymethyl-7,8-dihydropterin to form 6-hydroxymethyl-7,8-dihydropteridine pyrophosphate. This is the first step in a three-step pathway leading to 7,8-dihydrofolate. Bacterial HPPK (gene folK or sulD) [1] is a protein of 160 to 270 amino acids. In the lower eukaryote Pneumocystis carinii, HPPK is the central domain of a multifunctional folate synthesis enzyme (gene fas) [2]. As a signature for HPPK, a conserved region located in the central section of these enzymes was selected.

Consensus pattern [KRHD]-x-[GA]-[PSAE]-R-x(2)-D-[LIV]-D-[LIVM](2) Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROTNONE.

[1] Talarico T.L., Ray P.H., Dev I.K., Merrill B.M., Dallas W.S. J. Bacteriol. 174:5971-5977(1992).

[2] Volpes F., Dyer M., Scaife J.G., Darby G., Stammers D.K., Delves C.J. Gene 112:213-218(1992).

896. Metalloenzyme superfamily (Metalloenzyme)

This family includes phosphopentomutase Swiss:P07651 and 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, Swiss:P37689. This family is also related to alk_phosphatase [1]. The alignment contains the most conserved residues that are probably involved in metal binding and catalysis. Number of members: 34

[1] Galperin MY, Bairoch A, Koonin EV; Medline: 99180418 "A superfamily of metalloenzymes unifies phosphopentomutase and cofactor- independent phosphoglycerate mutase with alkaline phosphatases and sulfatases." Protein Sci 1998;7:1829-1835.

897. Penicillin amidase (Penicil_amidase)

Penicillin amidase or penicillin acylase EC:3.5.1.11 catalyses the hydrolysis of benzylpenicillin to phenylacetic acid and 6-aminopenicillanic acid (6-APA) a key intermediate in the the synthesis of penicillins [1]. Also in the family is cephalosporin acylase Swiss:P07662 and Swiss:P29958 aculeacin A acylase which are involved in the synthesis of related peptide antibiotics. Number of members: 13

[1] Verhaert RM, Riemens AM, van der Laan JM, van Duin J, Quax WJ; Medline: 97438505 "Molecular cloning and analysis of the gene encoding the thermostable penicillin G acylase from Alcaligenes faecalis. Appl Environ Microbiol 1997;63:3412-3418.

[2] Duggleby HJ, Tolley SP, Hill CP, Dodson EJ, Dodson G, Moody PC; Medline: 95115804 "Penicillin acylase has a single-amino-acid catalytic centre." Nature 1995;373:264-268.

898. Phosphoribosyl-AMP cyclohydrolase (PRA-CH)

This enzyme catalyses the third step in the histidine biosynthetic pathway. It requires Zn ions for activity. Number of members: 13

[1] D'Ordine RL, Klem TJ, Davisson VJ; Medline: 99129952 "N1-(5'-phosphoribosyl)adenosine-5'-monophosphate cyclohydrolase: purification and characterization of a unique metalloenzyme. Biochemistry 1999;38:1537-1546.

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899. Phosphoribosyl-ATP pyrophosphohydrolase (PRA-PH)

This enzyme catalyses the second step in the histidine biosynthetic pathway. Number of members: 32

[1] Keesey JK Jr, Bigelis R, Fink GR; Medline: 79216449 "The product of the his4 gene cluster in Saccharomyces cerevisiae. A trifunctional polypeptide." J Biol Chem 1979 Aug 10;254:7427-7433.

[2] Bruni CB, Carlomagno MS, Formisano S, Paolella G; Medline: 86310274 "Primary and secondary structural homologies between the HIS4 gene product of Saccharomyces cerevisiae and the hisIE and hisD gene products of Escherichia coli and Salmonella typhimurium." Mol Gen Genet 1986;203:389-396.

900. Prokaryotic membrane lipoprotein lipid attachment site (PstS)

In prokaryotes, membrane lipoproteins are synthesized with a precursor signal peptide, which is cleaved by a specific lipoprotein signal peptidase (signal peptidase II). The peptidase recognizes a conserved sequence and cuts upstream of a cysteine residue to which a glyceride-fatty acid lipid is attached [1]. Some of the proteins known to undergo such processing currently include (for recent listings see [1,2,3]):

- Major outer membrane lipoprotein (murein-lipoproteins) (gene lpp).
- Escherichia coli lipoprotein-28 (gene nlpA).
- Escherichia coli lipoprotein-34 (gene nlpB).
- Escherichia coli lipoprotein nlpC.
- Escherichia coli lipoprotein nlpD.
- Escherichia coli osmotically inducible lipoprotein B (gene osmB).
- Escherichia coli osmotically inducible lipoprotein E (gene osmE).
- Escherichia coli peptidoglycan-associated lipoprotein (gene pal).
- Escherichia coli rare lipoproteins A and B (genes rplA and rplB).
- Escherichia coli copper homeostasis protein cutF (or nlpE).
- Escherichia coli plasmids traT proteins.
- Escherichia coli Col plasmids lysis proteins.
- A number of Bacillus beta-lactamases.
- Bacillus subtilis periplasmic oligopeptide-binding protein (gene oppA).
- Borrelia burgdorferi outer surface proteins A and B (genes ospA and ospB).
- Borrelia hermsii variable major protein 21 (gene vmp21) and 7 (gene vmp7).
- Chlamydia trachomatis outer membrane protein 3 (gene omp3).
- Fibrobacter succinogenes endoglucanase cel-3.
- Haemophilus influenzae proteins Pal and Pcp.
- Klebsiella pullulunase (gene pulA).
- Klebsiella pullulunase secretion protein pulS.
- Mycoplasma hyorhinis protein p37.
- Mycoplasma hyorhinis variant surface antigens A, B, and C (genes vlpABC).
- Neisseria outer membrane protein H.8.
- Pseudomonas aeruginosa lipopeptide (gene lppL).
- Pseudomonas solanacearum endoglucanase egl.
- Rhodopseudomonas viridis reaction center cytochrome subunit (gene cytC).

- Rickettsia 17 Kd antigen.
- Shigella flexneri invasion plasmid proteins mxiJ and mxiM.
- Streptococcus pneumoniae oligopeptide transport protein A (gene amiA).
- Treponema pallidium 34 Kd antigen.
- Treponema pallidium membrane protein A (gene tmpA).
- Vibrio harveyi chitobiase (gene chb).
- Yersinia virulence plasmid protein yscJ.
- Halocyanin from Natrobacterium pharaonis [4], a membrane associated copper-binding protein. This is the first archaebacterial protein known to be modified in such a fashion).

From the precursor sequences of all these proteins, a consensus pattern was derived and a set of rules to identify this type of post-translational modification.

Consensus pattern {DERK}(6)-[LIVMFWSTAG](2)-[LIVMFYSTAGCQ]-[AGS]-C [C is the lipid attachment site] Additional rules: 1) The cysteine must be between positions 15 and 35 of the sequence in consideration. 2) There must be at least one Lys or one Arg in the first seven positions of the sequence. Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTsome 100 prokaryotic proteins. Some of them are not membrane lipoproteins, but at least half of them could be.

[1] Hayashi S., Wu H.C. J. Bioenerg. Biomembr. 22:451-471(1990).
[2] Klein P., Somorjai R.L., Lau P.C.K. Protein Eng. 2:15-20(1988).
[3] von Heijne G. Protein Eng. 2:531-534(1989).
[4] Mattar S., Scharf B., Kent S.B.H., Rodewald K., Oesterhelt D., Engelhard M. J. Biol. Chem. 269:14939-14945(1994).

901. Ribosome recycling factor (RRF)

The ribosome recycling factor (RRF / ribosome release factor) dissociates the ribosome from the mRNA after termination of translation, and is essential bacterial growth [1]. Thus ribosomes are "recycled" and ready for another round of protein synthesis. Number of members: 27

09595355 .091500

[1] Janosi L, Shimizu I, Kaji A; Medline: 94240115 "Ribosome recycling factor (ribosome releasing factor) is essential for bacterial growth." Proc Natl Acad Sci U S A 1994;91:4249-4253.

902. S-layer homology(SLH)

S-layers are paracrystalline mono-layered assemblies of (glyco)proteins which coat the surface of bacteria [1]. Several S-layer proteins and some other cell wall proteins contain one or more copies of a domain of about 50-60 residues, which has been called SLH (for S-layer homology) [2]. There is strong evidence that this domain serves as an anchor to the peptidoglycan [3]. The SLH domain has been found in:

- S-layer glycoprotein of Acetogenium kivui (3 copies).
- S-layer 125 Kd protein of Bacillus sphaericus (3 copies).
- S-layer protein of Bacillus anthracis (3 copies).
- S-layer protein of Bacillus licheniformis (3 copies).
- S-layer protein (HWP) from Bacillus brevis strain HPD31 (3 copies).
- Middle cell wall protein (MWP) from Bacillus brevis strain 47 (3 copies).
- S-layer protein (p100) of Thermus thermophilus (1 copy).
- Outer membrane protein Omp-alpha from Thermotoga maritima (1 copy).
- Cellulosome anchoring protein (gene ancA), outer layer protein B (OlpB) and a further potential cell surface glycoprotein from Clostridium thermocellum (3 copies; the first copy is missing its N-terminal third which is appended to the end of the third copy; may have arisen by circular permutation).
- Amylopullulanase (gene amyB) from Thermoanaerobacter thermosulfurogenes (3 copies)
- Amylopullulanase (gene aapT) from Bacillus strain XAL-601 (3 copies).
- Endoglucanase from Bacillus strain KSM-635 (3 copies).
- Exoglucanase (gene xynX) from Clostridium thermocellum (3 copies).
- Xylanase A (gene xynA) from Thermoanaerobacter saccharolyticum (2 copies; 3 copies if a frameshift is taken into account).
- Protein involved in butirosin production (ButB) from Bacillus circulans (2 incomplete copies; 3 copies if three frameshifts are taken into account).
- Two hypothetical proteins from Synechocystis strain PCC 6803 (1 copy each).

- A hypothetical protein with sequence similarity to amylopullulanases found 3' of amylase gene from Bacillus circulans (fragment of 1 copy; 3 copies if two frameshifts are taken into account).

SLH domains are found at the N- or C-termini of mature proteins. They occur in single copy followed by a predicted coiled coil domain, or in three contiguous copies. Structurally, the SLH domain is predicted to contain two alpha-helices flanking a beta strand. The SLH sequences are fairly divergent with an average identity of about 25%. It is however possible to build a sequence pattern that starts at the second position of the domain and that spans 3/4 of its length.

Consensus pattern[LVFYT]-x-[DA]-x(2,5)-[DNGSATPHY]-[FYWPDA]-x(4)-[LIV]-x(2)-[GTALV]-x(4,6)-[LIVFYC]-x(2)-G-x-[PGSTA]-x(2,3)-[MFYA]-x- [PGAV]-x(3,10)-[LIVMA]-[STKR]-[RY]-x-[EQ]-x-[STALIVM] Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROTNONE.

[1] Beveridge T.J. Curr. Opin. Struct. Biol. 4:204-212(1994).
[2] Lupas A., Engelhardt H., Peters J., Santarius U., Volker S., Baumeister W. J. Bacteriol. 176:1224-1233(1994).
[3] Lemaire M., Ohayon H., Gounon P., Fujino T., Beguin P. J. Bacteriol. 177:2451-2459(1995).

903. Queuine tRNA-ribosyltransferase (TGT)

This is a family of queuine tRNA-ribosyltransferases EC:2.4.2.29, also known as tRNA-guanine transglycosylase and guanine insertion enzyme. Queuine tRNA-ribosyltransferase modifies tRNAs for asparagine, aspartic acid, histidine and tyrosine with queuine. It catalyses the exchange of guanine-34 at the wobble position with 7-aminomethyl-7-deazaguanine, and the addition of a cyclopentenediol moiety to 7-aminomethyl-7-deazaguanine-34 tRNA; giving a hypermodified base queuine in the wobble position [1,2].The aligned region contains a zinc binding motif C-x-C-x2-C-x29-H, and important tRNA and 7-aminomethyl-7deazaguanine binding residues [1]. Number of members: 27

09595325.071902

[1] Romier C, Reuter K, Suck D, Ficner R; Medline: 96256303 “Crystal structure of tRNA-guanine transglycosylase: RNA modification by base exchange.” EMBO J 1996;15:2850-2857.

[2] Garcia GA, Koch KA, Chong S; Medline: 93287116 “tRNA-guanine transglycosylase from Escherichia coli. Overexpression, purification and quaternary structure.” J Mol Biol 1993;231:489-497.

904. ThiC Family (ThiC)

ThiC is found within the thiamine biosynthesis operon. ThiC is involved in pyrimidine biosynthesis [2]. ThiC catalyzes the substitution of the pyrophosphate of 2-methyl-4-amino-5-hydroxymethylpyrimidine pyrophosphate by 4-methyl-5-(beta-hydroxyethyl)thiazole phosphate to yield thiamine phosphate [3]. Number of members: 12

[1] Vander Horn PB, Backstrom AD, Stewart V, Begley TP; Medline: 93163063 “Structural genes for thiamine biosynthetic enzymes (thiCEFGH) in Escherichia coli K-12.” J Bacteriol 1993;175:982-992.

[2] Begley TP, Downs DM, Ealick SE, McLafferty FW, Van Loon AP, Taylor S, Campobasso N, Chiu HJ, Kinsland C, Reddick JJ, Xi J; Medline: 99311269 “Thiamin biosynthesis in prokaryotes.” Arch Microbiol 1999;171:293-300.

[3] Zhang Y, Taylor SV, Chiu HJ, Begley TP; Medline: 97284509 “Characterization of the Bacillus subtilis thiC operon involved in thiamine biosynthesis.” J Bacteriol 1997;179:3030-3035.

905. Putative tRNA binding domain (tRNA_bind)

This domain is found in prokaryotic methionyl-tRNA synthetases, prokaryotic phenylalanyl tRNA synthetases the yeast GU4 nucleic-binding protein (G4p1 or p42, ARC1) [2], human tyrosyl-tRNA synthetase [1], and endothelial-monocyte activating polypeptide II. G4p1 binds specifically to tRNA form a complex with methionyl-tRNA synthetases [2]. In human tyrosyl-tRNA synthetase this domain may direct tRNA to the active site of the enzyme [2]. This domain may perform a common function in tRNA aminoacylation [1]. Number of members: 12

09552351.061502

[1] Kleeman TA, Wei D, Simpson KL, First EA; Medline: 97306356 "Human tyrosyl-tRNA synthetase shares amino acid sequence homology with a putative cytokine." J Biol Chem 1997;272:14420-14425.

[2] Simos G, Segref A, Fasiolo F, Hellmuth K, Shevchenko A, Mann M, Hurt EC; Medline: 97050848 "The yeast protein Arc1p binds to tRNA and functions as a cofactor for the methionyl-and glutamyl-tRNA synthetases." EMBO J 1996;15:5437-5448.

906. UbiA prenyltransferase family signature (UbiA)

The following prenyltransferases are evolutionary related [1,2]:

- Bacterial 4-hydroxybenzoate octaprenyltransferase (gene ubiA).
- Yeast mitochondrial para-hydroxybenzoate--polyprenyltransferase (gene COQ2).
- Protoheme IX farnesyltransferase (heme O synthase) from yeast and mammals (gene COX10) and from bacteria (genes cyoE or ctaB).

These proteins probably contain seven transmembrane segments. The best conserved region is located in a loop between the second and third of these segments and was used as a signature pattern.

Consensus pattern N-x(3)-[DE]-x(2)-[LIF]-D-x(2)-[VM]-x-R-[ST]-x(2)-R-x(4)-G Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROTNONE.

[1] Melzer M., Heide L. Biochim. Biophys. Acta 1212:93-102(1994).

[2] Mogi T., Saiki K., Anraku Y. Mol. Microbiol. 14:391-398(1994).

907. Uncharacterized protein family UPF0044 signature (UPF0044)

The following uncharacterized proteins have been shown [1] to be highly similar:

- Bacillus subtilis hypothetical protein yqeI.
- Escherichia coli hypothetical protein yhbY and HI1333, the corresponding Haemophilus influenzae protein.

- Methanococcus jannaschii hypothetical protein MJ0652.

These are small proteins of 10 to 15 Kd. They can be picked up in the database by the following pattern. This pattern is located in the N-terminal part of these proteins.

Consensus pattern L-[ST]-x(3)-K-x(3)-[KR]-[SGA]-x-[GA]-H-x-L-x-P-[LIV]-x(2)- [LIV]-[GA]-x(2)-G Sequences known to belong to this class detected by the patternALL.

908. ATP synthase (C/AC39) subunit (vATP-synt_AC39)

This family includes the AC39 subunit from vacuolar ATP synthase Swiss:P32366 [1], and the C subunit from archaebacterial ATP synthase [2]. The family also includes subunit C from the Sodium transporting ATP synthase from Enterococcus hirae Swiss:P43456 [3]. Number of members: 12

[1] Bauerle C, Ho MN, Lindorfer MA, Stevens TH; Medline: 93286119 “The Saccharomyces cerevisiae VMA6 gene encodes the 36-kDa subunit of the vacuolar H(+)-ATPase membrane sector.” J Biol Chem 1993;268:12749-12757.

[2] Wilms R, Freiberg C, Wegerle E, Meier I, Mayer F, Muller V; Medline: 96324968 “Subunit structure and organization of the genes of the A1A0 ATPase from the Archaeon Methanosarcina mazei Go1.” J Biol Chem 1996;271:18843-18852.

[3] Takase K, Kakinuma S, Yamato I, Konishi K, Igarashi K, Kakinuma Y; Medline: 94209269 “Sequencing and characterization of the ntp gene cluster for vacuolar- type Na(+)-translocating ATPase of Enterococcus hirae.” J Biol Chem 1994;269:11037-11044.

909. ATP synthase (E/31 kDa) subunit (vATP-synt_E)

This family includes the vacuolar ATP synthase E subunit [1], as well as the archaebacterial ATP synthase E subunit [2]. Number of members: 24

[1] Foury F; Medline: 91009356 “The 31-kDa polypeptide is an essential subunit of the vacuolar ATPase in Saccharomyces cerevisiae.” J Biol Chem 1990;265:18554-18560.

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[2] Wilms R, Freiberg C, Wegerle E, Meier I, Mayer F, Muller V; Medline: 96324968 “Subunit structure and organization of the genes of the A1A0 ATPase from the Archaeon Methanosarcina mazei Go1.” J Biol Chem 1996;271:18843-18852.

910. (WW)

The WW domain [1-4,E1] (also known as rsp5 or WWP) has been originally discovered as a short conserved region in a number of unrelated proteins, among them dystrophin, the gene responsible for Duchenne muscular dystrophy. The domain, which spans about 35 residues, is repeated up to 4 times in some proteins. It has been shown [5] to bind proteins with particular proline- motifs, [AP]-P-P-[AP]-Y, and thus resembles somewhat SH3 domains. It appears to contain beta-strands grouped around four conserved aromatic positions; generally Trp. The name WW or WWP derives from the presence of these Trp as well as that of a conserved Pro. It is frequently associated with other domains typical for proteins in signal transduction processes.

Proteins containing the WW domain are listed below.

- Dystrophin, a multidomain cytoskeletal protein. Its longest alternatively spliced form consists of an N-terminal actin-binding domain, followed by 24 spectrin-like repeats, a cysteine-rich calcium-binding domain and a C- terminal globular domain. Dystrophin form tetramers and is thought to have multiple functions including involvement in membrane stability, transduction of contractile forces to the extracellular environment and organization of membrane specialization. Mutations in the dystrophin gene lead to muscular dystrophy of Duchenne or Becker type. Dystrophin contains one WW domain C-terminal of the spectrin-repeats.
- Utrophin, a dystrophin-like protein of unknown function.
- Vertebrate YAP protein is a substrate of an unknown serine kinase. It binds to the SH3 domain of the Yes oncoprotein via a proline-rich region. This protein appears in alternatively spliced isoforms, containing either one or two WW domains [6].
- Mouse NEDD-4 plays a role in the embryonic development and differentiation of the central nervous system. It contains 3 WW modules followed by a HECT domain. The human ortholog contains 4 WW domains, but the third WW domain is probably spliced resulting in an alternate NEDD-4 protein with only 3 WW modules [3].

- Yeast RSP5 is similar to NEDD-4 in its molecular organization. It contains an N-terminal C2 domain (see <PDOC00380>, followed by a histidine-rich region, 3 WW domains and a HECT domain.
- Rat FE65, a transcription-factor activator expressed preferentially in liver. The activator domain is located within the N-terminal 232 residues of FE65, which also contain the WW domain.
- Yeast ESS1/PTF1, a putative peptidyl prolyl cis-trans isomerase from family ppiC (see <PDOC00840>). A related protein, dodo (gene dod) exists in Drosophila and in mammals (gene PIN1).
- Tobacco DB10 protein. The WW domain is located N-terminal to the region with similarity to ATP-dependent RNA helicases.
- IQGAP, a human GTPase activating protein acting on ras. It contains an N- terminal domain similar to fly muscle mp20 protein and a C-terminal ras GTPase activator domain.
- Yeast pre-mRNA processing protein PRP40, Caenorhabditis elegans ZK1098.1 and fission yeast SpAC13C5.02 are related proteins with similarity to MYO2- type myosin, each containing two WW-domains at the N-terminus.
- Caenorhabditis elegans hypothetical protein C38D4.5, which contains one WW module, a PH domain (see <PDOC50003>) and a C-terminal phosphatidylinositol 3-kinase domain.
- Yeast hypothetical protein YFL010c.

For the sensitive detection of WW domains, a profile was developed which spans the whole homology region as well as a pattern.

Consensus pattern W-x(9,11)-[VFY]-[FYW]-x(6,7)-[GSTNE]-[GSTQCR]-[FYW]-x(2)-P
Sequences known to belong to this class detected by the pattern ALL. Other sequence(s) detected in SWISS-PROT8. Sequences known to belong to this class detected by the profileALL.

[1] Bork P., Sudol M. Trends Biochem. Sci. 19:531-533(1994).
[2] Andre B., Springael J.Y. Biochem. Biophys. Res. Commun. 205:1201-1205(1994).
[3] Hofmann K.O., Bucher P. FEBS Lett. 358:153-157(1995).
[4] Sudol M., Chen H.I., Bougeret C., Einbond A., Bork P. FEBS Lett. 369:67-71(1995).
[5] Chen H.I., Sudol M. Proc. Natl. Acad. Sci. U.S.A. 92:7819-7823(1995).

[6] Sudol M., Bork P., Einbond A., Kastury K., Druck T., Negrini M., Huebner K., Lehman D. J. Biol. Chem. 270:14733-14741(1995).

911. Xeroderma pigmentosum (XP) [1] (XPG_1)

Xeroderma pigmentosum (XP) [1] is a human autosomal recessive disease, characterized by a high incidence of sunlight-induced skin cancer. People's skin cells with this condition are hypersensitive to ultraviolet light, due to defects in the incision step of DNA excision repair. There are a minimum of seven genetic complementation groups involved in this pathway: XP-A to XP-G. The defect in XP-G can be corrected by a 133 Kd nuclear protein called XPG (or XPGC) [2].

XPG belongs to a family of proteins [2,3,4,5,6] that are composed of two main subsets:

- Subset 1, to which belongs XPG, RAD2 from budding yeast and rad13 from fission yeast. RAD2 and XPG are single-stranded DNA endonucleases [7,8]. XPG makes the 3'incision in human DNA nucleotide excision repair [9].
- Subset 2, to which belongs mouse and human FEN-1, rad2 from fission yeast, and RAD27 from budding yeast. FEN-1 is a structure-specific endonuclease.

In addition to the proteins listed in the above groups, this family also includes:

- Fission yeast exo1, a 5'->3' double-stranded DNA exonuclease that could act in a pathway that corrects mismatched base pairs.
- Yeast EXO1 (DHS1), a protein with probably the same function as exo1.
- Yeast DIN7.

Sequence alignment of this family of proteins reveals that similarities are largely confined to two regions. The first is located at the N-terminal extremity (N-region) and corresponds to the first 95 to 105 amino acids. The second region is internal (I-region) and found towards the C-terminus; it spans about 140 residues and contains a highly conserved core of 27 amino acids that includes a conserved pentapeptide (E-A-[DE]-A-[QS]). It is possible that the conserved acidic residues are involved in the catalytic mechanism of DNA excision repair in XPG. The amino acids linking the N- and I-regions are not conserved; indeed, they are largely absent from proteins belonging to the second subset.

Two signature patterns were developed for these proteins. The first corresponds to the central part of the N-region, the second to part of the I-region and includes the putative catalytic core pentapeptide.

Consensus pattern [VI]-[KRE]-P-x-[FYIL]-V-F-D-G-x(2)-[PIL]-x-[LVC]-K Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

Consensus pattern [GS]-[LIVM]-[PER]-[FYS]-[LIVM]-x-A-P-x-E-A-[DE]-[PAS]- [QS]-[CLM] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

[1] Tanaka K., Wood R.D. Trends Biochem. Sci. 19:83-86(1994).
[2] Scherly D., Nouspikel T., Corlet J., Ucla C., Bairoch A., Clarkson S.G. Nature 363:182-185(1993).
[3] Carr A.M., Sheldrick K.S., Murray J.M., Al-Harithy R., Watts F.Z., Lehmann A.R. Nucleic Acids Res. 21:1345-1349(1993).
[4] Murray J.M., Tavassoli M., Al-Harithy R., Sheldrick K.S., Lehmann A.R., Carr A.M., Watts F.Z. Mol. Cell. Biol. 14:4878-4888(1994).
[5] Harrington J.J., Lieber M.R. Genes Dev. 8:1344-1355(1994).
[6] Szankasi P., Smith G.R. Science 267:1166-1169(1995).
[7] Habraken Y., Sung P., Prakash L., Prakash S. Nature 366:365-368(1993).
[8] O'Donovan A., Scherly D., Clarkson S.G., Wood R.D. J. Biol. Chem. 269:15965-15968(1994).
[9] O'Donovan A., Davies A.A., Moggs J.G., West S.C., Wood R.D. Nature 371:432-435(1994).

912. 5-formyltetrahydrofolate cyclo-ligase (5-FTHF_cyc-lig)

5-formyltetrahydrofolate cyclo-ligase or methenyl-THF synthetase EC:6.3.3.2 catalyses the interchange of 5-formyltetrahydrofolate (5-FTHF) to 5-10-methenyltetrahydrofolate, this requires ATP and Mg2+ [1]. 5-FTHF is used in chemotherapy where it is clinically known as Leucovorin [2].

Number of members: 23

[1] Dayan A, Bertrand R, Beauchemin M, Chahla D, Mamo A, Filion M, Skup D, Massie B, Jolivet J; Medline: 96096540 "Cloning and characterization of the human 5,10-methenyltetrahydrofolate synthetase-encoding cDNA." Gene 1995;165:307-311.

[2] Maras B, Stover P, Valiante S, Barra D, Schirch V; Medline: 94308074 "Primary structure and tetrahydropteroylglutamate binding site of rabbit liver cytosolic 5,10-methenyltetrahydrofolate synthetase." J Biol Chem 1994;269:18429-18433.

09535605 .071901

913. Cytosolic long-chain acyl-CoA thioester hydrolase (Acyl-CoA_hydro)

This family consist of various cytosolic long-chain acyl-CoA thioester hydrolases including human and rat [1,2]. The aligned region is repeated with in the sequence of human and rat cytosolic long-chain acyl-CoA thioester hydrolases of this family. Long-chain acyl-CoA hydrolases hydrolyse palmitoyl-CoA to CoA and palmitate, they also catalyse the hydrolysis of other long chain fatty acyl-CoA thioesters. Long-chain acyl-CoA hydrolases are present in all living organisms and they may provide a mechanism for the control of lipid metabolism [1].

Number of members: 24

[1]Yamada J, Furihata T, Iida N, Watanabe T, Hosokawa M, Satoh T, Someya A, Nagaoka I, Suga T; Medline: 97236308 "Molecular cloning and expression of cDNAs encoding rat brain and liver cytosolic long-chain acyl-CoA hydrolases." Biochem Biophys Res Commun 1997;232:198-203.

[2] Broustas CG, Larkins LK, Uhler MD, Hajra AK; Medline: 96209964 "Molecular cloning and expression of cDNA encoding rat brain cytosolic acyl-coenzyme A thioester hydrolase." J Biol Chem 1996;271:10470-10476.

914. Agglutinin

Lectin (probable mannose binding)

Members of this family are plant lectins. Many if not all are mannose specific.

Number of members: 87

[1] Wright CS, Hester G; Medline: 97094989 “The 2.0 A structure of a cross-linked complex between snowdrop lectin and a branched mannopentaose: evidence for two unique binding modes." Structure 1996;4:1339-1352.

915. (ANF_RECEPTORS)

Natriuretic peptides are hormones involved in the regulation of fluid and electrolyte homeostasis. These hormones stimulate the intracellular production of cyclic GMP as a second messenger.

Currently, three types of natriuretic peptide receptors are known [1,2]. Two express guanylate cyclase activity: GC-A (or ANP-A) which seems specific to atrial natriuretic peptide (ANP), and GC-B (or ANP-B) which seems to be stimulated more effectively by brain natriuretic peptide (BNP) than by ANP. The third receptor (ANP-C) is probably responsible for the clearance of ANP from the circulation and does not play a role in signal transduction.

GC-A and GC-B are plasma membrane-bound proteins that share the following topology: an N-terminal extracellular domain which acts as the ligand binding region, then a transmembrane domain followed by a large cytoplasmic C- terminal region that can be subdivided into two domains: a protein kinase-like domain (see <PDOC00100>) that appears important for proper signalling and a guanylate cyclase catalytic domain (see <PDOC00425>). The topology of ANP-C is different: like GC-A and -B it possesses an extracellular ligand-binding region and a transmembrane domain, but its cytoplasmic domain is very short.

A pattern was developed from the ligand-binding region of natriuretic peptide receptors based on a highly conserved region located in the N-terminal part of the domain.

Consensus patternG-P-x-C-x-Y-x-A-A-x-V-x-R-x(3)-H-W Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

09565351.061501

[1] Garbers D.L. New Biol. 2:499-504(1990).

[2] Schulz S., Chinkers M., Garbers D.L. FASEB J. 2:2026-2035(1989).

916. (Apocytochrome)
Cytochrome c family heme-binding site signature

In proteins belonging to cytochrome c family [1], the heme group is covalently attached by thioether bonds to two conserved cysteine residues. The consensus sequence for this site is Cys-X-X-Cys-His and the histidine residue is one of the two axial ligands of the heme iron. This arrangement is shared by all proteins known to belong to cytochrome c family, which presently includes cytochromes c, c', c1 to c6, c550 to c556, cc3/Hmc, cytochrome f and reaction center cytochrome c.

Consensus patternC-{CPWHF}-{CPWR}-C-H-{CFYW} Sequences known to belong to this class detected by the patternALL, except for four cytochrome c's which lack the first thioether bond. Other sequence(s) detected in SWISS-PROT454.

Note: some cytochrome c's have more than a single bound heme groupc4 has 2, c7 has 3, c3 has 4, the reaction center has 4, and cc3/Hmc has 16 !

[1] Mathews F.S. Prog. Biophys. Mol. Biol. 45:1-56(1985).

917. ATP-synt_A-c. ATP synthase Alpha chain, C terminal
[1] Medline: 94344236. Structure at 2.8 A resolution of F1-ATPase from bovine heart mitochondria. Abrahams JP, Leslie AG, Lutter R, Walker JE; Nature 1994;370:621-628.
Number of members: 125

918. (Basic)
Myc-type, 'helix-loop-helix' dimerization domain signature
HELIX_LOOP_HELIX

A number of eukaryotic proteins, which probably are sequence specific DNA- binding proteins that act as transcription factors, share a conserved domain of 40 to 50 amino acid residues. It has been proposed [1] that this domain is formed of two amphipathic helices joined by a variable length linker region that could form a loop. This 'helix-loop-helix' (HLH) domain mediates protein dimerization and has been found in the proteins listed below [2,3,E1,E2]. Most of these proteins have an extra basic region of about 15 amino acid residues that is adjacent to the HLH domain and specifically binds to DNA. They are refered as basic helix-loop-helix proteins (bHLH), and are classified in two groups: class A (ubiquitous) and class B (tissue-specific). Members of the bHLH family bind variations on the core sequence 'CANNTG', also refered to as the E-box motif. The homo- or heterodimerization mediated by the HLH domain is independent of, but necessary for DNA binding, as two basic regions are required for DNA binding activity. The HLH proteins lacking the basic domain (Emc, Id) function as negative regulators since they form heterodimers, but fail to bind DNA. The hairy-related proteins (hairy, E(spl), deadpan) also repress transcription although they can bind DNA. The proteins of this subfamily act together with co-repressor proteins, like groucho, through their C-terminal motif WRPW.

- The myc family of cellular oncogenes [4], which is currently known to contain four members: c-myc [E3], N-myc, L-myc, and B-myc. The myc genes are thought to play a role in cellular differentiation and proliferation.
- Proteins involved in myogenesis (the induction of muscle cells). In mammals MyoD1 (Myf-3), myogenin (Myf-4), Myf-5, and Myf-6 (Mrf4 or herculin), in birds CMD1 (QMF-1), in Xenopus MyoD and MF25, in Caenorhabditis elegans CeMyoD, and in Drosophila nautilus (nau).
- Vertebrate proteins that bind specific DNA sequences ('E boxes') in various immunoglobulin chains enhancers: E2A or ITF-1 (E12/pan-2 and E47/pan-1), ITF-2 (tcf4), TFE3, and TFEB.
- Vertebrate neurogenic differentiation factor 1 that acts as differentiation factor during neurogenesis.
- Vertebrate MAX protein, a transcription regulator that forms a sequence- specific DNA-binding protein complex with myc or mad.
- Vertebrate Max Interacting Protein 1 (MXI1 protein) which acts as a transcriptional repressor and may antagonize myc transcriptional activity by competing for max.

- Proteins of the bHLH/PAS superfamily which are transcriptional activators. In mammals, AH receptor nuclear translocator (ARNT), single-minded homologs (SIM1 and SIM2), hypoxia-inducible factor 1 alpha (HIF1A), AH receptor (AHR), neuronal pas domain proteins (NPAS1 and NPAS2), endothelial pas domain protein 1 (EPAS1), mouse ARNT2, and human BMAL1. In drosophila, single-minded (SIM), AH receptor nuclear translocator (ARNT), trachealess protein (TRH), and similar protein (SIMA).
- Mammalian transcription factors HES, which repress transcription by acting on two types of DNA sequences, the E box and the N box.
- Mammalian MAD protein (max dimerizer) which acts as transcriptional repressor and may antagonize myc transcriptional activity by competing for max.
- Mammalian Upstream Stimulatory Factor 1 and 2 (USF1 and USF2), which bind to a symmetrical DNA sequence that is found in a variety of viral and cellular promoters.
- Human lyl-1 protein; which is involved, by chromosomal translocation, in T- cell leukemia.
- Human transcription factor AP-4.
- Mouse helix-loop-helix proteins MATH-1 and MATH-2 which activate E box- dependent transcription in collaboration with E47.
- Mammalian stem cell protein (SCL) (also known as tal1), a protein which may play an important role in hemopoietic differentiation. SCL is involved, by chromosomal translocation, in stem-cell leukemia.
- Mammalian proteins Id1 to Id4 [5]. Id (inhibitor of DNA binding) proteins lack a basic DNA-binding domain but are able to form heterodimers with other HLH proteins, thereby inhibiting binding to DNA.
- Drosophila extra-macrochaetae (emc) protein, which participates in sensory organ patterning by antagonizing the neurogenic activity of the achaete- scute complex. Emc is the homolog of mammalian Id proteins.
- Human Sterol Regulatory Element Binding Protein 1 (SREBP-1), a transcriptional activator that binds to the sterol regulatory element 1 (SRE-1) found in the flanking region of the LDLR gene and in other genes.
- Drosophila achaete-scute (AS-C) complex proteins T3 (l'sc), T4 (scute), T5 (achaete) and T8 (asense). The AS-C proteins are involved in the determination of the neuronal precursors in the peripheral nervous system and the central nervous system.
- Mammalian homologs of achaete-scute proteins, the MASH-1 and MASH-2 proteins.
- Drosophila atonal protein (ato) which is involved in neurogenesis.

- Drosophila daughterless (da) protein, which is essential for neurogenesis and sex-determination.
- Drosophila deadpan (dpn), a hairy-like protein involved in the functional differentiation of neurons.
- Drosophila delilah (dei) protein, which is plays an important role in the differentiation of epidermal cells into muscle.
- Drosophila hairy (h) protein, a transcriptional repressor which regulates the embryonic segmentation and adult bristle patterning.
- Drosophila enhancer of split proteins E(spl), that are hairy-like proteins active during neurogenesis. also act as transcriptional repressors.
- Drosophila twist (twi) protein, which is involved in the establishment of germ layers in embryos.
- Maize anthocyanin regulatory proteins R-S and LC.
- Yeast centromere-binding protein 1 (CPF1 or CBF1). This protein is involved in chromosomal segregation. It binds to a highly conserved DNA sequence, found in centromers and in several promoters.
- Yeast INO2 and INO4 proteins.
- Yeast phosphate system positive regulatory protein PHO4 which interacts with the upstream activating sequence of several acid phosphatase genes.
- Yeast serine-rich protein TYE7 that is required for ty-mediated ADH2 expression.
- Neurospora crassa nuc-1, a protein that activates the transcription of structural genes for phosphorus acquisition.
- Fission yeast protein esc1 which is involved in the sexual differentiation process.

The schematic representation of the helix-loop-helix domain is shown here: xxxxxxxxxxxxxxxxxxxxxxxxx-------------------xxxxxxxxxxxxxxxxxxxxxxxx Amphipathic helix 1 Loop Amphipathic helix 2

The signature pattern that had been developed to detect this domain spans completely the second amphipathic helix.

Consensus pattern[DENSTAP]-[KR]-[LIVMAGSNT]-{FYWCPHKR}-[LIVMT]-[LIVM]-x(2)-[STAV]-[LIVMSTACKR]-x-[VMFYH]-[LIVMTA]-{P}-{P}- [LIVMRKHQ]

Sequences known to belong to this class detected by the pattern the majority but far from all. Other sequence(s) detected in SWISS-PROT135.

[1] Murre C., McCaw P.S., Baltimore D. Cell 56:777-783(1989).
[2] Garrel J., Campuzano S. BioEssays 13:493-498(1991).
[3] Kato G.J., Dang C.V. FASEB J. 6:3065-3072(1992).
[4] Krause M., Fire A., Harrison S.W., Priess J., Weintraub H. Cell 63:907-919(1990).
[5] Riechmann V., van Cruechten I., Sablitzky F. Nucleic Acids Res. 22:749-755(1994).

919. (Beta-lactamase)
Beta-lactamases classes -A, -C, and -D active site

Beta-lactamases (EC 3.5.2.6) [1,2] are enzymes which catalyze the hydrolysis of an amide bond in the beta-lactam ring of antibiotics belonging to the penicillin/cephalosporin family. Four kinds of beta-lactamase have been identified [3]. Class-B enzymes are zinc containing proteins whilst class -A, C and D enzymes are serine hydrolases. The three classes of serine beta-lactamases are evolutionary related and belong to a superfamily [4] that also includes DD-peptidases and a variety of other penicillin-binding proteins (PBP's). All these proteins contain a Ser-x-x-Lys motif, where the serine is the active site residue. Although clearly homologous, the sequences of the three classes of serine beta-lactamases exhibit a large degree of variability and only a small number of residues are conserved in addition to the catalytic serine.

Since a pattern detecting all serine beta-lactamases would also pick up many unrelated sequences, it was decided to provide specific patterns, centered on the active site serine, for each of the three classes.

Consensus pattern [FY]-x-[LIVMFY]-x-S-[TV]-x-K-x(4)-[AGLM]-x(2)-[LC] [S is the active site residue] Sequences known to belong to this class detected by the patternALL class-A beta-lactamases. Other sequence(s) detected in SWISS-PROT7.

Consensus pattern F-E-[LIVM]-G-S-[LIVMG]-[SA]-K [The first S is the active site residue] Sequences known to belong to this class detected by the patternALL class-C beta-lactamases. Other sequence(s) detected in SWISS-PROTNONE.

Consensus pattern [PA]-x-S-[ST]-F-K-[LIV]-[PAL]-x-[STA]-[LI] [S is the active site residue] Sequences known to belong to this class detected by the patternALL class-D beta-lactamases. Other sequence(s) detected in SWISS-PROTNONE.

[1] Ambler R.P. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 289:321-331(1980).
[2] Pastor N., Pinero D., Valdes A.M., Soberon X. Mol. Microbiol. 4:1957-1965(1990).
[3] Bush K. Antimicrob. Agents Chemother. 33:259-263(1989).
[4] Joris B., Ghuysen J.-M., Dive G., Renard A., Dideberg O., Charlier P., Frere J.M., Kelly J.A., Boyington J.C., Moews P.C., Knox J.R. Biochem. J. 250:313-324(1988).

09595326 . 061600

920. Biotin protein ligase (BPL)

Biotin is covalently attached at the active site of certain enzymes that transfer carbon dioxide from bicarbonate to organic acids to form cellular metabolites. Biotin protein ligase (BPL) is the enzyme responsible for attaching biotin to a specific lysine at the active site of biotin enzymes. Each organism probably has only one BPL. Biotin attachment is a two step reaction that results in the formation of an amide linkage between the carboxyl group of biotin and the epsilon-amino group of the modified lysine [2].
Number of members: 26

[1] Wilson KP, Shewchuk LM, Brennan RG, Otsuka AJ, Matthews BW; Medline: 93028443 “Escherichia coli biotin holoenzyme synthetase/bio repressor crystal structure delineates the biotin- and DNA-binding domains.” Proc Natl Acad Sci USA 1992;89:9257-9261.
[2] Chapman-Smith A, Cronan JE Jr; Medline: 10470036 “The enzymatic biotinylation of proteins: a post-translational modification of exceptional specificity.” Trends Biochem Sci 1999;24:359-363.

921. (BRCA2_repeat)

The alignment covers only the most conserved region of the repeat. Respiratory-chain NADH dehydrogenase 30 Kd subunit signature

[1] Bork P, Blomberg N, Nilges M; Medline: 96241568 "Internal repeats in the BRCA2 protein sequence." Nat Genet 1996;13:22-23.

Number of members: 63

922. (C6)

This domain of unknown function is found in the C. elegans protein Swiss:Q19522. It is presumed to be an extracellular domain. The C6 domain contains six conserved cysteine residues in most copies of the domain. However some copies of the domain are missing cysteine residues 1 and 3 suggesting that these form a disulphide bridge.

Number of members: 23

923. Cadherin cytoplasmic region (Cadherin_C_term)

Cadherins are vital in cell-cell adhesion during tissue differentiation. Cadherins are linked to the cytoskeleton by catenins. Catenins bind to the cytoplasmic tail of the cadherin. Cadherins cluster to form foci of homophilic binding units. A key determinant to the strength of the binding that it is mediated by cadherins is the juxtamembrane region of the cadherin. This region induces clustering and also binds to the protein p120ctn [1].

Number of members: 59

[1] Yap AS, Niessen CM, Gumbiner BM; Medline: 98234411 "The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening, and interaction with p120ctn." J Cell Biol 1998;141:779-789.

[2] Barth AI, Nathke IS, Nelson WJ; Medline: 97471931 "Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways." Curr Opin Cell Biol 1997;9:683-690.

09559382.091502

[3] Braga VM, Machesky LM, Hall A, Hotchin NA; Medline: 97327766 "The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts." J Cell Biol 1997;137:1421-1431.

924. Clathrin propeller repeat (Clathrin_propel)

Clathrin is the scaffold protein of the basket-like coat that surrounds coated vesicles. The soluble assembly unit, a triskelion, contains three heavy chains and three light chains in an extended three-legged structure. Each leg contains one heavy and one light chain. The N-terminus of the heavy chain is known as the globular domain, and is composed of seven repeats which form a beta propeller [1].

Number of members: 61

[1] ter Haar E, Musacchio A, Harrison SC, Kirchhausen T; Medline: 99043510 "Atomic structure of clathrin: a beta propeller terminal domain joins an alpha zigzag linker." Cell. 1998;95:563-573.

925. Respiratory-chain NADH dehydrogenase 30 Kd subunit signature (complex1_30Kd)

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 30 Kd (in mammals) which has been found to be:

- Nuclear encoded, as a precursor form with a transit peptide in mammals, and in Neurospora crassa.
- Mitochondrial encoded in Paramecium (protein P1), and in the slime mold Dictyostelium discoideum (ORF 209).
- Chloroplast encoded in various higher plants (ORF 159). It is also present in bacteria:
- In the cyanobacteria Synechocystis strain PCC 6803 (gene ndhJ).
- Subunit C of Escherichia coli NADH-ubiquinone oxidoreductase (gene nuoC).
- Subunit NQO5 of Paracoccus denitrificans NADH-ubiquinone oxidoreductase.

09559356.061502

This protein, in its mature form, consists of from 157 to 266 amino acid residues. The best conserved region is located in the C-terminal section and can be used as a signature pattern.

Consensus pattern E-R-E-x(2)-[DE]-[LIVMFY](2)-x(6)-[HK]-x(3)-[KRP]-x-[LIVM]-[LIVMYS] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).
[2]Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).

09565305.051503

926. Respiratory-chain NADH dehydrogenase 49 Kd subunit signature (complex1_49Kd)

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there is one with a molecular weight of 49 Kd (in mammals), which is the third largest subunit of complex I and is a component of the iron-sulfur (IP) fragment of the enzyme. It seems to bind a 4Fe-4S iron-sulfur cluster. The 49 Kd subunit has been found to be:

- Nuclear encoded, as a precursor form with a transit peptide in mammals, and in Neurospora crassa.
- Mitochondrial encoded in protozoan such as Paramecium (ORF 400), Leishmania and Trypanosoma (MURF 3).
- Chloroplast encoded in various higher plants (ORF 392).

The 49 Kd subunit is highly similar to [3,4]:

- Subunit D of Escherichia coli NADH-ubiquinone oxidoreductase (gene nuoD).
- Subunit NQO4 of Paracoccus denitrificans NADH-ubiquinone oxidoreductase.
- Subunit 5 of Escherichia coli formate hydrogenlyase (gene hycE).
- Subunit G of Escherichia coli hydrogenase-4 (gene hyfG).

A highly conserved region was seleceted as signature pattern, located in the N-terminal section of this subunit.

Consensus pattern [LIVMH]-H-[RT]-[GA]-x-E-K-[LIVMTN]-x-E-x-[KRQ] Sequences known to belong to this class detected by the patternALL.

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).
[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).
[3] Fearnley I.M., Walker J.E. Biochim. Biophys. Acta 1140:105-134(1992).
[4] Weidner U., Geier S., Ptock A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-122(1993).

927. (COX2)

Cytochrome c oxidase (EC 1.9.3.1) [1,2] is an oligomeric enzymatic complex which is a component of the respiratory chain and is involved in the transfer of electrons from cytochrome c to oxygen. In eukaryotes this enzyme complex is located in the mitochondrial inner membrane; in aerobic prokaryotes it is found in the plasma membrane. The enzyme complex consists of 3-4 subunits (prokaryotes) to up to 13 polypeptides (mammals).

Subunit 2 (CO II) transfers the electrons from cytochrome c to the catalytic subunit 1. It contains two adjacent transmembrane regions in its N-terminus and the major part of the protein is exposed to the periplasmic or to the mitochondrial intermembrane space, respectively. CO II provides the substrate- binding site and contains a copper center called Cu(A), probably the primary acceptor in cytochrome c oxidase. An exception is the corresponding subunit of the cbb3-type oxidase which lacks the copper A redox-center. Several bacterial CO II have a C-terminal extension that contains a covalently bound heme c.

It has been shown [3,4] that nitrous oxide reductase (EC 1.7.99.6) (gene nosZ) of Pseudomonas has sequence similarity in its C-terminus to CO II. This enzyme is part of the bacterial respiratory system which is activated under anaerobic conditions in the presence of nitrate or nitrous oxide. NosZ is a periplasmic homodimer that contains a dinuclear copper center, probably located in a 3- dimensional fold similar to the cupredoxin-like fold that has been suggested for the copper-binding site of CO II [3].

The dinuclear purple copper center is formed by 2 histidines and 2 cysteines [5]. This region was used as a signature pattern. The conserved valine and the conserved methionine are said to be involved in stabilizing the copper-binding fold by interacting with each other.

Consensus pattern V-x-H-x(33,40)-C-x(3)-C-x(3)-H-x(2)-M [The two C's and two H's are copper ligands] Sequences known to belong to this class detected by the patternALL, except for Paramecium primaurelia as well as in some plants where the pattern ends with Thr; an RNA editing event at this position could change this Thr to Met.

Note: cytochrome cbb(3) subunit 2 does not belong to this family.

[1] Capaldi R.A., Malatesta F., Darley-Usmar V.M. Biochim. Biophys. Acta 726:135-148(1983).

[2] Garcia-Horsman J.A., Barquera B., Rumbley J., Ma J., Gennis R.B. J. Bacteriol. 176:5587-5600(1994).

[3] van der Oost J., Lappalainen P., Musacchio A., Warne A., Lemieux L., Rumbley J., Gennis R.B., Aasa R., Pascher T., Malmstrom B.G., Saraste M. EMBO J. 11:3209-3217(1992).

[4] Zumft W.G., Dreutsch A., Loechelt S., Cuypers H., Friedrich B., Schneider B. Eur. J. Biochem. 208:31-40(1992).

928. Cytochrome C assembly protein (CytC_asm)

This family consists of various proteins involved in cytochrome c assembly from mitochondria and bacteria; CycK from Rhizobium[3], CcmC from E. coli and Paracoccus denitrificans [2,1] and orf240 from wheat mitochondria [4]. The members of this family are probably integral membrane proteins with six predicted transmembrane helices. It has been proposed that members of this family comprise a membrane component of an ABC (ATP binding cassette) transporter complex. It is also proposed that this transporter is necessary for transport of some component needed for cytochrome c assembly. One member CycK contains a putative heme-binding motif [3], orf240 also contains a putative heme-binding motif and is a proposed ABC transporter with c-type heme as its proposed substrate [4].

However it seems unlikely that all members of this family transport heme nor c-type apocytochromes because CcmC in the putative CcmABC transporter transports neither [1].

Number of members: 67

[1] Page D, Pearce DA, Norris HA, Ferguson SJ; Medline: 97195802 "The Paracoccus denitrificans ccmA, B and C genes: cloning and sequencing, and analysis of the potential of their products to form a haem or apo-c-type cytochrome transporter. MICROBIOLOGY 1997;143:563-576.

[2] Thoeny-meyer L, Fischer F, Kunzler P, Ritz D, Hennecke H; Medline: 95362656 "Escherichia coli genes required for cytochrome c maturation." J. BACTERIOL 1995;177:4321-4326.

[3] Delgado MJ, Yeoman KH, Wu G, Vargas C, Davies A, Poole RK, Johnston AWB, Downie JA; Medline: 95394794 "Characterization of the cycHJKL genes involved in cytochrome c biogenesis and symbiotic nitrogen fixation in Rhizobium leguminosarum." J. BACTERIOL 1995;177:4927-4934.

[4] Bonnard G, Grienenberger JM; Medline: 95124303 "A gene proposed to encode a transmembrane domain of an ABC transporter is expressed in wheat mitochondria." MOL. GEN. GENET 1995;246:91-99.

929. Cytochrome b559 subunits heme-binding site signature (cytochr_b559)

Cytochrome b559 [1] is an essential component of photosystem II complex from oxygenic photosynthetic organisms. It is an integral thylakoid membrane protein composed of two subunits, alpha (gene psbE) and beta (gene psbF), each of which contains a histidine residue located in a transmembrane region. The two histidines coordinate the heme iron of cytochrome b559.

The region around the heme-binding residue of both subunits is very similar and can be used as a signature pattern.

Consensus pattern[LIV]-x-[ST]-[LIVF]-R-[FYW]-x(2)-[IV]-H-[STGA]-[LIV]- [STGA]-[IV]-P [H is the heme iron ligand] Sequences known to belong to this class detected by the patternALL. Other sequence(s) detected in SWISS-PROTNONE.

[1] Pakrasi H.B., de Ciechi P., Whitmarsh J. EMBO J. 10:1619-1627(1991).

930. Cytochrome b/b6 signatures (Cytochrome_b)

In the mitochondrion of eukaryotes and in aerobic prokaryotes, cytochrome b is a component of respiratory chain complex III (EC 1.10.2.2) - also known as the bc1 complex or ubiquinol-cytochrome c reductase. In plant chloroplasts and cyanobacteria, there is a analogous protein, cytochrome b6, a component of the plastoquinone-plastocyanin reductase (EC 1.10.99.1), also known as the b6f complex.

Cytochrome b/b6 [1,2] is an integral membrane protein of approximately 400 amino acid residues that probably has 8 transmembrane segments. In plants and cyanobacteria, cytochrome b6 consists of two subunits encoded by the petB and petD genes. The sequence of petB is colinear with the N-terminal part of mitochondrial cytochrome b, while petD corresponds to the C-terminal part. Cytochrome b/b6 non-covalently binds two heme groups, known as b562 and b566. Four conserved histidine residues are postulated to be the ligands of the iron atoms of these two heme groups.

Apart from regions around some of the histidine heme ligands, there are a few conserved regions in the sequence of b/b6. The best conserved of these regions includes an invariant P-E-W triplet which lies in the loop that separates the fifth and sixth transmembrane segments. It seems to be important for electron transfer at the ubiquinone redox site - called Qz or Qo (where o stands for outside) - located on the outer side of the membrane.

A schematic representation of the structure of cytochrome b/b6 is shown below.

```
+---Fe-b562----+ | +---Fe-b566--|-+ | | | |
xxxxxxxxxxxHxHxxxxxxxxxxxxxHxHxxxxxxxxxxPEWxxxxxxxxxxxxxxxxxx <--------------
---Cytochrome-b--------------------------> <----Cytochrome-b6-petB--------><--Cytochrome-
b6-petD----->
```

Two signature patterns were developed for cytochrome b/b6. The first includes the first conserved histidine of b/b6, which is a heme b562 ligand; the second includes the conserved PEW triplet.

Consensus pattern [DENQ]-x(3)-G-[FYWMQ]-x-[LIVMF]-R-x(2)-H [H is a heme b562 ligand] Sequences known to belong to this class detected by the patternALL, except for 5 sequences.

Consensus pattern P-[DE]-W-[FY]-[LFY](2) Sequences known to belong to this class detected by the patternALL, except for Odocoileus hemionus (mule deer) and Paramecium tetraurelia cytochrome b.

[1] Howell N. J. Mol. Evol. 29:157-169(1989).
[2] Esposti M.D., de Vries S., Crimi M., Ghelli A., Patarnello T., Meyer A. Biochim. Biophys. Acta 1143:243-271(1993).

931. Phorbol esters / diacylglycerol binding domain (DAG_PE-bind)

Diacylglycerol (DAG) is an important second messenger. Phorbol esters (PE) are analogues of DAG and potent tumor promoters that cause a variety of physiological changes when administered to both cells and tissues. DAG activates a family of serine/threonine protein kinases, collectively known as protein kinase C (PKC) [1]. Phorbol esters can directly stimulate PKC. The N- terminal region of PKC, known as C1, has been shown [2] to bind PE and DAG in a phospholipid and zinc-dependent fashion. The C1 region contains one or two copies (depending on the isozyme of PKC) of a cysteine-rich domain about 50 amino-acid residues long and essential for DAG/PE-binding. Such a domain has also been found in the following proteins:

- Diacylglycerol kinase (EC 2.7.1.107) (DGK) [3], the enzyme that converts DAG into phosphatidate. It contains two copies of the DAG/PE-binding domain in its N-terminal section. At least five different forms of DGK are known in mammals.
- N-chimaerin. A brain specific protein which shows sequence similarities with the BCR protein at its C-terminal part and contains a single copy of the DAG/PE-binding domain at its N-terminal part. It has been shown [4,5] to be able to bind phorbol esters.

- The raf/mil family of serine/threonine protein kinases. These protein kinases contain a single N-terminal copy of the DAG/PE-binding domain.
- The unc-13 protein from Caenorhabditis elegans. Its function is not known but it contains a copy of the DAG/PE-binding domain in its central section and has been shown to bind specifically to a phorbol ester in the presence of calcium [6].
- The vav oncogene. Vav was generated by a genetic rearrangement during gene transfer assays. Its expression seems to be restricted to cells of hematopoeitic origin. Vav seems [5,7] to contain a DAG/PE-binding domain in the central part of the protein.
- The Drosophila GTPase activating protein rotund.

The DAG/PE-binding domain binds two zinc ions; the ligands of these metal ions are probably the six cysteines and two histidines that are conserved in this domain. A signature pattern was developed that spans completely the DAG/PE domain.

Consensus pattern H-x-[LIVMFYW]-x(8,11)-C-x(2)-C-x(3)-[LIVMFC]-x(5,10)- C-x(2)-C-x(4)-[HD]-x(2)-C-x(5,9)-C [All the C and H are involved in binding Zinc] Sequences known to belong to this class detected by the pattern ALL, except a few DGK's.

[1] Azzi A., Boscoboinik D., Hensey C. Eur. J. Biochem. 208:547-557(1992).
[2] Ono Y., Fujii T., Igarashi K., Kuno T., Tanaka C, Kikkawa U., Nishizuka Y. Proc. Natl. Acad. Sci. U.S.A. 86:4868-4871(1989).
[3] Sakane F., Yamada K., Kanoh H., Yokoyama C., Tanabe T. Nature 344:345-348(1990).
[4] Ahmed S., Kozma R., Monfries C., Hall C., Lim H.H., Smith P., Lim L. Biochem. J. 272:767-773(1990).
[5] Ahmed S., Kozma R., Lee J., Monfries C., Harden N., Lim L. Biochem. J. 280:233-241(1991).
[6] Ahmed S., Maruyama I.N., Kozma R., Lee J., Brenner S., Lim L. Biochem. J. 287:995-999(1992).
[7] Boguski M.S., Bairoch A., Attwood T.K., Michaels G.S. Nature 358:113-113(1992).

932. 3-dehydroquinate synthase (DHQ_synthase)

[1] Barten R, Meyer TF; Medline: 98273626 “Cloning and characterisation of the Neisseria gonorrhoeae aroB gene.” Mol Gen Genet 1998;258:34-44.

[2] Hawkins AR, Lamb HK; Medline: 96048023 “The molecular biology of multidomain proteins. Selected examples.” Eur J Biochem 1995;232:7-18.

The 3-dehydroquinate synthase EC:4.6.1.3 domain is present in isolation in various bacterial 3-dehydroquinate synthases and also present as a domain in the pentafunctional AROM polypeptide Swiss:P07547 [2]. 3-dehydroquinate (DHQ) synthase catalyses the formation of dehydroquinate (DHQ) and orthophosphate from 3-deoxy-D-arabino heptulosonic 7 phosphate [1]. This reaction is part of the shikimate pathway which is involved in the biosynthesis of aromatic amino acids.

Number of members: 25

09583505.051702

933. Dihydrofolate reductase signature (DiHfolate_red)

Dihydrofolate reductases (EC 1.5.1.3) [1] are ubiquitous enzymes which catalyze the reduction of folic acid into tetrahydrofolic acid. They can be inhibited by a number of antagonists such as trimethroprim and methotrexate which are used as antibacterial or anticancerous agents. A signature pattern was derived from a region in the N-terminal part of these enzymes, which includes a conserved Pro-Trp dipeptide; the tryptophan has been shown [2] to be involved in the binding of substrate by the enzyme.

Consensus pattern[LVAGC]-[LIF]-G-x(4)-[LIVMF]-P-W-x(4,5)-[DE]-x(3)-[FYIV]-x(3)-[STIQ] Sequences known to belong to this class detected by the patternALL, except for type II bacterial, plasmid-encoded, dihydrofolate reductases which do not belong to the same class of enzymes.

[1] Harpers' Review of Biochemistry, Lange, Los Altos (1985).

[2] Bolin J.T., Filman D.J., Matthews D.A., Hamlin R.C., Kraut J. J. Biol. Chem. 257:13650-13662(1982).

934. (DIL)

[1] Ponting CP; Medline: 95397417 “AF-6/cno: neither a kinesin nor a myosin, but a bit of both.” Trends Biochem Sci 1995;20:265-266.

Number of members: 31

935. (DNA_gyraseB_C)

DNA topoisomerase II signature (cross-reference = TOPOISOMERASE_II)

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break. Topoisomerase II is found in phages, archaebacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits (the product of genes 39, 52 and 60). In prokaryotes and in archaebacteria the enzyme, known as DNA gyrase, consists of two subunits (genes gyrA and gyrB [E2]). In some bacteria, a second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes parC and parE). In eukaryotes, type II topoisomerase is a homodimer.

There are many regions of sequence homology between the different subtypes of topoisomerase II. The relation between the different subunits is shown in the following representation:

```
<----------------About-1400-residues----------------------->
[----------Protein 39-*-----][----Protein 52----] Phage T4
[----------gyrB-------*-----][--------gyrA-----------------] Prokaryote II
 Archaebacteria
[----------parE-------*-----][--------parD-----------------] Prokaryote IV
[--------------------*-----------------------------------] Eukaryote and ASF
```

'*': Position of the pattern.

As a signature pattern for this family of proteins, a region was selected that contains a highly conserved pentapeptide. The pattern is located in gyrB, in parE, and in protein 39 of phage T4 topoisomerase.

Consensus pattern [LIVMA]-x-E-G-[DN]-S-A-x-[STAG] Sequences known to belong to this class detected by the pattern ALL.

[1] Sternglanz R. Curr. Opin. Cell Biol. 1:533-535(1990).
[2] Bjornsti M.-A. Curr. Opin. Struct. Biol. 1:99-103(1991).
[3] Sharma A., Mondragon A. Curr. Opin. Struct. Biol. 5:39-47(1995).
[4] Roca J. Trends Biochem. Sci. 20:156-160(1995).

936. (DNA_topoisoIIV)
DNA topoisomerase II signature (cross-reference = TOPOISOMERASE_II)

DNA topoisomerase I (EC 5.99.1.2) [1,2,3,4,E1] is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers. Type II topoisomerases are ATP-dependent and act by passing a DNA segment through a transient double-strand break. Topoisomerase II is found in phages, archaebacteria, prokaryotes, eukaryotes, and in African Swine Fever virus (ASF). In bacteriophage T4 topoisomerase II consists of three subunits (the product of genes 39, 52 and 60). In prokaryotes and in archaebacteria the enzyme, known as DNA gyrase, consists of two subunits (genes gyrA and gyrB [E2]). In some bacteria, a second type II topoisomerase has been identified; it is known as topoisomerase IV and is required for chromosome segregation, it also consists of two subunits (genes parC and parE). In eukaryotes, type II topoisomerase is a homodimer.

There are many regions of sequence homology between the different subtypes of topoisomerase II. The relation between the different subunits is shown in the following representation:

<----------------About-1400-residues----------------------->
[----------Protein 39-*-----][----Protein 52----] Phage T4
[----------gyrB-------*-----][--------gyrA----------------] Prokaryote II Archaebacteria

[----------parE-------*-----][--------parD-----------------] Prokaryote IV
[--------------------*-----------------------------------] Eukaryote and ASF
'*': Position of the pattern.

As a signature pattern for this family of proteins, a region was selected that contains a highly conserved pentapeptide. The pattern is located in gyrB, in parE, and in protein 39 of phage T4 topoisomerase.

Consensus pattern [LIVMA]-x-E-G-[DN]-S-A-x-[STAG] Sequences known to belong to this class detected by the patternALL.

[1] Sternglanz R. Curr. Opin. Cell Biol. 1:533-535(1990).
[2] Bjornsti M.-A. Curr. Opin. Struct. Biol. 1:99-103(1991).
[3] Sharma A., Mondragon A. Curr. Opin. Struct. Biol. 5:39-47(1995).
[4] Roca J. Trends Biochem. Sci. 20:156-160(1995).

09525325.021500

937. Prolyl oligopeptidase family serine active site (DPPIV_N_term)

The prolyl oligopeptidase family [1,2,3] consist of a number of evolutionary related peptidases whose catalytic activity seems to be provided by a charge relay system similar to that of the trypsin family of serine proteases, but which evolved by independent convergent evolution. The known members of this family are listed below.

- Prolyl endopeptidase (EC 3.4.21.26) (PE) (also called post-proline cleaving enzyme). PE is an enzyme that cleaves peptide bonds on the C-terminal side of prolyl residues. The sequence of PE has been obtained from a mammalian species (pig) and from bacteria (Flavobacterium meningosepticum and Aeromonas hydrophila); there is a high degree of sequence conservation between these sequences.
- Escherichia coli protease II (EC 3.4.21.83) (oligopeptidase B) (gene prtB) which cleaves peptide bonds on the C-terminal side of lysyl and argininyl residues.
- Dipeptidyl peptidase IV (EC 3.4.14.5) (DPP IV). DPP IV is an enzyme that removes N-terminal dipeptides sequentially from polypeptides having unsubstituted N-termini provided that the penultimate residue is proline.

- Yeast vacuolar dipeptidyl aminopeptidase A (DPAP A) (gene: STE13) which is responsible for the proteolytic maturation of the alpha-factor precursor.
- Yeast vacuolar dipeptidyl aminopeptidase B (DPAP B) (gene: DAP2).
- Acylamino-acid-releasing enzyme (EC 3.4.19.1) (acyl-peptide hydrolase). This enzyme catalyzes the hydrolysis of the amino-terminal peptide bond of an N-acetylated protein to generate a N-acetylated amino acid and a protein with a free amino-terminus.

A conserved serine residue has experimentally been shown (in E.coli protease II as well as in pig and bacterial PE) to be necessary for the catalytic mechanism. This serine, which is part of the catalytic triad (Ser, His, Asp), is generally located about 150 residues away from the C-terminal extremity of these enzymes (which are all proteins that contains about 700 to 800 amino acids).

Consensus pattern D-x(3)-A-x(3)-[LIVMFYW]-x(14)-G-x-S-x-G-G-[LIVMFYW](2) [S is the active site residue] Sequences known to belong to this class detected by the pattern ALL, except for yeast DPAP A.

Note: these proteins belong to families S9A/S9B/S9C in the classification of peptidases [4,E1].

[1] Rawlings N.D., Polgar L., Barrett A.J. Biochem. J. 279:907-911(1991).
[2] Barrett A.J., Rawlings N.D. Biol. Chem. Hoppe-Seyler 373:353-360(1992).
[3] Polgar L., Szabo E. Biol. Chem. Hoppe-Seyler 373:361-366(1992).
[4] Rawlings N.D., Barrett A.J. Meth. Enzymol. 244:19-61(1994).

938. Deoxyhypusine synthase (DS)

Eukaryotic initiation factor 5A (eIF-5A) contains an unusual amino acid, hypusine [N epsilon-(4-aminobutyl-2-hydroxy)lysine]. The first step in the post-translational formation of hypusine is catalysed by the enzyme deoxyhypusine synthase (DS) EC:1.1.1.249. The modified version of eIF-5A, and DS, are required for eukaryotic cell proliferation [1].

Number of members: 9

[1] Liao DI, Wolff EC, Park MH, Davies DR; Medline: 98154315 “Crystal structure of the NAD complex of human deoxyhypusine synthase: an enzyme with a ball-and-chain mechanism for blocking the active site.” Structure 1998;6:23-32.

939. (DUF21)

Many of the sequences in this family are annotated as hemolysins, however this is due to a similarity to Swiss:Q54318 that does not contain this domain. This domain is found in the N-terminus of the proteins adjacent to two intracellular CBS domains CBS.
Number of members: 42

940. (DUF59)

This family includes prokaryotic proteins of unknown function. The family also includes PhaH Swiss:O84984 from Pseudomonas putida. PhaH forms a complex with PhaF Swiss:O84982, PhaG Swiss:O84983 and PhaI Swiss:O84985, which hydroxylates phenylacetic acid to 2-hydroxyphenylacetic acid [1]. So members of this family may all be components of ring hydroxylating complexes.
Number of members: 15

[1] Olivera ER, Minambres B, Garcia B, Muniz C, Moreno MA, Ferrandez A, Diaz E, Garcia JL, Luengo JM; Medline: 98263372 “Molecular characterization of the phenylacetic acid catabolic pathway in Pseudomonas putida U: the phenylacetyl-CoA catabolon.” Proc Natl Acad Sci U S A 1998;95:6419-6424.

941. (DUF82)

The protein contains four conserved cysteines that may be involved in metal binding or disulphide bridges.
Number of members: 4

942. Riboflavin kinase / FAD synthetase (FAD_Synth)

This family consists part of the bifunctional enzyme riboflavin kinase / FAD synthetase. These enzymes have both ATP:riboflavin 5'-phospho transferase and ATP:FMN-adenylyltransferase activitys [1]. They catalyse the 5'-phosphorylation of riboflavin to FMN and the adenylylation of FMN to FAD [1].

CAUTION: It is not clear if this region of the enzymes catalyses either or both of the enzymatic reactions.

Number of members: 27

[1] Manstein DJ, Pai EF; Medline: 87057286 "Purification and characterization of FAD synthetase from Brevibacterium ammoniagenes." J Biol Chem 1986;261:16169-16173.

09565205.051500

943. [2Fe-2S] binding domain (fer2_2)

[1] Romao MJ, Archer M, Moura I, Moura JJ, LeGall J, Engh R, Schneider M, Hof P, Huber R; Medline: 96072968 "Crystal structure of the xanthine oxidase-related aldehyde oxido-reductase from D. gigas." Science 1995;270:1170-1176.

Number of members: 53

944. Filovirus glycoprotein (Filo_glycop)

This family includes an extracellular region from the envelope glycoprotein of Ebola and Marburg viruses. This region is also produced as a separate transcript that gives rise to a non-structural, secreted glycoprotein, which is produced in large amounts and has an unknown function [1]. Processing of this protein may be involved in viral pathogenicity [2].

Number of members: 23

[1] Volchkov VE, Feldmann H, Volchkova VA, Klenk HD; Medline: 98245155 "Processing of the Ebola virus glycoprotein by the proprotein convertase furin." Proc Natl Acad Sci U S A 1998;95:5762-5767.

[2] Sanchez A, Trappier SG, Mahy BW, Peters CJ, Nichol ST; Medline: 96195018 "The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing." Proc Natl Acad Sci U S A 1996;93:3602-3607.

945. Frataxin-like domain (Frataxin_Cyay)

This family contains proteins that have a domain related to the globular C-terminus of Frataxin the protein that is mutated in Friedreich's ataxia. This domain is found in a family of bacterial proteins. The function of this domain is currently unknown.

Number of members: 12

[1] Gibson TJ, Koonin EV, Musco G, Pastore A, Bork P; Medline: 97084946 "Friedreich's ataxia protein: phylogenetic evidence for mitochondrial dysfunction." Trends Neurosci 1996;19:465-468.

09525026.061502

946. (GAF)

Domain present in phytochromes and cGMP-specific phosphodiesterases.

Number of members: 296

[1] Aravind L, Ponting CP; Medline: 98094688 "The GAF domain: an evolutionary link between diverse phototransducing proteins." Trends Biochem Sci 1997;22:458-459.

947. Galaptin signature (Gal-bind_lectin)

All vertebrates synthesize soluble galactoside-binding lectins [1,2,3] (also known as galectins, galaptins or S-lectin). These carbohydrate-binding proteins are developmentally regulated. Although their exact physiological role is not yet clear they seem to be involved in differentiation, cellular regulation and tissue construction. The sequence of galactoside-binding lectins from electric eel (electrolectin), conger eel (congerin), chicken and a number of mammalian species is known. These lectins are proteins of about 130 to 140 amino acid residues (14 Kd to 16 Kd).

A number of other proteins are known to belong to this family:

- Galectin-3 (also known as MAC-2 antigen; CBP-35 or IgE-binding protein), a 35 Kd lectin which binds immunoglobulin E and which is composed of two domains: a N-terminal domain that consist of tandem repeats of a glycine/ proline-rich sequence and a C-terminal galaptin domain.
- Galectin-4 [4], which is composed of two galaptin domains.
- Galectin-5.
- Galectin-7 [5], a keratinocyte protein which could be involved in cell-cell and/or cell-matrix interactions necessary for normal growth control.
- Galectin-8 [6], which is composed of two galaptin domains.
- Galectin-9 [7], which is composed of two galaptin domains.
- Human eosinophil lysophospholipase (EC 3.1.1.5) [8] (Charcot-Leyden crystal protein), a protein that may have both an enzymatic and a lectin activities. It forms hexagonal bipyramidal crystals in tissues and secretions from sites of eosinophil-associated inflammation.
- Caenorhabditis elegans 32 Kd lactose-binding lectin [9]. This lectin is composed of two galaptin domains.
- Caenorhabditis elegans lec-7 and lec-8.

One of the conserved regions of these lectins contains a tryptophan that has been shown [10] to be essential to the binding of galactosides. This region was used as a signature pattern for these proteins.

Consensus patternW-[GEK]-x-[EQ]-x-[KRE]-x(3,6)-[PCTF]-[LIVMF]-[NQEGSKV]-x-[GH]-x(3)-[DENKHS]-[LIVMFC] [W binds carbohydrate] Sequences known to belong to this class detected by the pattern ALL, except for pig galectin 4.

[1] Barondes S.H., Gitt M.A., Leffler H., Cooper D.N.W. Biochimie 70:1627-1632(1988).

[2] Hirabayashi J., Kasai K.-I. J. Biochem. 104:1-4(1988).

[3] Barondes S.H., Castronovo V., Cooper D.N.W., Cummings R.D., Drickamer K., Feizi T., Gitt M.A., Hirabayashi J., Hughes C., Kasai K.-I., Leffler H., Liu F.-T., Lotan R., Mercurio A.M., Monsigny M., Pillair S., Poirer F., Raz A., Rigby P.W.J., Rini J.M., Wang J.L. Cell 76:597-598(1994).

09555325.061401

[4] Oda Y., Herrmann J., Gitt M., Turck C.W., Burlingame A.L., Barondes S.H., Leffler H. J. Biol. Chem. 268:5929-5939(1993).

[5] Madsen P., Rasmussen H.H., Flint T., Gromov P., Kruse T.A., Honore B., Vorum H., Celis J.E. J. Biol. Chem. 270:5823-5829(1995).

[6] Hadari Y.R., Paz K., Dekel R., Mestrovic T., Accili D., Zick Y. J. Biol. Chem. 270:3447-3453(1995).

[7] Wada J., Kanwar Y.S. J. Biol. Chem. 272:6078-6086(1997).

[8] Ackerman S.J., Corrette S.E., Rosenberg H.F., Bennett J.C., Mastrianni D.M., Nicholson-Weller A., Weller P.F., Chin D.T., Tenen D.G. J. Immunol. 150:456-468(1993).

[9] Hirabayashi J., Satoh M., Kasai K.-I. J. Biol. Chem. 267:15485-15490(1992).

[10] Abbott W.M., Feizi T. J. Biol. Chem. 266:5552-5557(1991).

948. (GARS) Phosphoribosylglycinamide synthetase signature (phosphoribosylamine glycine ligase)

PROSITE: PDOC00164; cross-reference(s): PS00184

[1] catalyzes the second step in the de novo biosynthesis of purine, the ATP-dependent addition of 5-phosphoribosylamine to glycine to form 5'phosphoribosylglycinamide.

In bacteria GARS is a monofunctional enzyme (encoded by the purD gene), in of a bifunctional enzyme (encoded by the ADE5,7 gene), in higher eukaryotes it is part, with AIRS and with phosphoribosylglycinamide formyltransferase (GART) of a trifunctional enzyme (GARS-AIRS-GART).

The sequence of GARS is well conserved. A highly conserved octapeptide was selected as a signature pattern.

Consensus patternR-F-G-D-P-E-x-[QM]
Sequences known to belong to this class detected by the patternALL.

[1]Aiba A., Mizobuchi K. J. Biol. Chem. 264:21239-21246(1989).

949. GLTT - GLTT repeat (12 copies)

This short repeat of unknown function is found in multiple copies in several C. elegans proteins. The repeat is five residues long and consists of XGLTT where X can be any amino acid. Number of members: 34.

950. Glu_synthase - Conserved region in glutamate synthase

This family represents a region of the glutamate synthase protein. This region is expressed as a seperate subunit in the glutamate synthase alpha subunit from archaebacteria, or part of a large multidomain enzyme in other organisms. The aligned region of these proteins contains a putative FMN binding site and Fe-S cluster. Number of members: 44.

[1] Medline: 97082505. Sequence of the GLT1 gene from Saccharomyces cerevisiae reveals the domain structure of yeast glutamate synthase. Filetici P, Martegani MP, Valenzuela L, Gonzalez A, Ballario P; Yeast 1996;12:1359-1366.

09535305.051501

951. (Glyco_hydro_2) Glycosyl hydrolases family 2 signatures

GLYCOSYL_HYDROL_F2_1; PS00608; GLYCOSYL_HYDROL_F2_2

It has been shown [1,2,E1] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

-Beta-galactosidases (EC 3.2.1.23) from bacteria such as Escherichia coli (genes lacZ and ebgA), Clostridium acetobutylicum, Clostridium thermosulfurogenes, Klebsiella pneumoniae, Lactobacillus delbrueckii, or Streptococcus thermophilus and from the fungi Kluyveromyces lactis.

-Beta-glucuronidase (EC 3.2.1.31) from Escherichia coli (gene uidA) and from mammals.

One of the conserved regions in these enzymes is centered on a conserved glutamic acid residue which has been shown [3], in Escherichia coli lacZ, to be the general acid/base catalyst in the active site of the enzyme. This region has been used as a signature pattern. A highly conserved region located some sixty residues upstream from the active site glutamate has been selected as a second signature pattern.

Consensus pattern N-x-[LIVMFYWD]-R-[STACN](2)-H-Y-P-x(4)-[LIVMFYWS](2)-x(3)-[DN]-x(2)-G-[LIVMFYW](4) Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [DENQLF]-[KRVW]-N-[HRY]-[STAPPV]-[SAC]-[LIVMFS](3)-W-[GS]-x(2,3)-N-E [E is the active site residue] Sequences known to belong to this class detected by the pattern ALL, except for Rhizobium meliloti lacZ.

[1]Henrissat B. Biochem. J. 280:309-316(1991).
[2]Schroeder C.J., Robert C., Lenzen G., McKay L.L., Mercenier A. J. Gen. Microbiol. 137:369-380(1991).
[3]Gebler J.C., Aebersold R., Withers S.G. J. Biol. Chem. 267:11126-11130(1992).

952. (Glyco_hydro_3) Glycosyl hydrolases family 3 active site
PROSITE: PDOC00621. PROSITE cross-reference(s)PS00775; GLYCOSYL_HYDROL_F3

It has been shown [1,2] that the following glycosyl hydrolases can be, on the basis of sequence similarities, classified into a single family:

-Beta glucosidases (EC 3.2.1.21) from the fungi Aspergillus wentii (A-3), Hansenula anomala, Kluyveromyces fragilis, Saccharomycopsis fibuligera,(BGL1 and BGL2), Schizophyllum commune and Trichoderma reesei (BGL1).

-Beta glucosidases from the bacteria Agrobacterium tumefaciens (Cbg1), Butyrivibrio fibrisolvens (bglA), Clostridium thermocellum (bglB), Escherichia coli (bglX), Erwinia chrysanthemi (bgxA) and Ruminococcus albus.

-Alteromonas strain O-7 beta-hexosaminidase A (EC 3.2.1.52).

-Bacillus subtilis hypothetical protein yzbA.

-Escherichica coli hypothetical protein ycfO and HI0959, the corresponding Haemophilus influenzae protein.

One of the conserved regions in these enzymes is centered on a conserved aspartic acid residue which has been shown [3], in Aspergillus wentii beta-glucosidase A3, to be implicated in the catalytic mechanism. This region was used as a signature pattern.

Consensus pattern[LIVM](2)-[KR]-x-[EQK]-x(4)-G-[LIVMFT]-[LIVT]-[LIVMF]-[ST]-D-x(2)-[SGADNI] [D is the active site residue]
Sequences known to belong to this class detected by the patternALL.

[1]Henrissat B. Biochem. J. 280:309-316(1991).
[2]Castle L.A., Smith K.D., Morris R.O. J. Bacteriol. 174:1478-1486(1992).

[3]Bause E., Legler G. Biochim. Biophys. Acta 626:459-465(1980).

953. GP120 - Envelope glycoprotein GP120

The entry of HIV requires interaction of viral GP120 with Swiss:P01730 and a chemokine receptor on the cell surface. Number of members: 17891

[1]Medline: 98303379. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA; Nature 1998;393:648-659.

954. (GSPII_E) Bacterial type II secretion system protein E signature

PROSITE: PDOC00567. PROSITE cross-reference(s) PS00662; T2SP_E

A number of bacterial proteins, some of which are involved in a general secretion pathway (GSP) for the export of proteins (also called the type II pathway) [1,2], have been found to be evolutionary related. These proteins are listed below:

-The 'E' protein from the GSP operon of: Aeromonas (gene exeE); Erwinia (gene outE); Escherichia coli (gene yheG); Klebsiella pneumoniae (gene pulE); Pseudomonas aeruginosa (gene xcpR); Vibrio cholerae (gene epsE) and Xanthomonas campestris (gene xpsE).

-Agrobacterium tumefaciens Ti plasmid virB operon protein 11. This protein is required for the transfer of T-DNA to plants.

-Bacillus subtilis comG operon protein 1 which is required for the uptake of DNA by competent Bacillus subtilis cells.

-Aeromonas hydrophila tapB, involved in type IV pilus assembly.

-Pseudomonas protein pilB, which is essential for the formation of the pili.

-Pseudomonas aeruginosa protein twitching mobility protein pilT.

-Neisseria gonorrhoeae type IV pilus assembly protein pilF.

-Vibrio cholerae protein tcpT, which is involved in the biosynthesis of the tcp pilus.

-Escherichia coli protein hofB (hopB).

-Escherichia coli hypothetical protein ygcB.

-Escherichia coli hypothetical protein yggR.

These proteins have from 344 (pilT and virB11) to 568 (tapB) amino acids, they are probably cytoplasmically located and, on the basis of the presence of a conserved P-loop

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region (see <PDOC00017>), probably bind ATP. A region that overlaps the 'B' motif of ATP-binding proteins was selected as a signature pattern.

Consensus pattern[LIVM]-R-x(2)-P-D-x-[LIVM](3)-G-E-[LIVM]-R-D
Sequences known to belong to this class detected by the patternALL, except for ygcB.

[1]Salmond G.P.C., Reeves P.J. Trends Biochem. Sci. 18:7-12(1993).
[2]Hobbs M., Mattick J.S. Mol. Microbiol. 10:233-243(1993).

955. (guanylate_cyc) Guanylate cyclases signature
PROSITE: PDOC00425. PROSITE cross-reference(s) PS00452;
GUANYLATE_CYCLASES Guanylate cyclases (EC 4.6.1.2) [1 to 4] catalyze the formation of cyclic GMP (cGMP) from GTP. cGMP acts as an intracellular messenger, activating cGMP dependent kinases and regulating CGMP-sensitive ion channels. The role of cGMP as a second messenger in vascular smooth muscle relaxation and retinal photo-transduction is well established. Guanylate cyclase is found both in the soluble and particular fraction of eukaryotic cells. The soluble and plasma membrane-bound forms differ in structure, regulation and other properties.

Most currently known plasma membrane-bound forms are receptors for small polypeptides. The topology of such proteins is the following: they have a N-terminal extracellular domain which acts as the ligand binding region, then a transmembrane domain, followed by a large cytoplasmic C-terminal region that can be subdivided into two domains: a protein kinase-like domain that appears important for proper signalling and a cyclase catalytic domain. This topology is schematically represented below.

```
+----------------------xxxxx---------------------+---------------+
| Ligand-binding    XXXXX Protein Kinase like | Cyclase  |
+----------------------xxxxx---------------------+---------------+
 Extracellular    Transmembrane    Cytoplasmic
```

The known guanylate cyclase receptors are:
-The sea-urchins receptors for speract and resact, which are small peptides that stimulate sperm motility and metabolism.

-The receptors for natriuretic peptides (ANF). Two forms of ANF receptors with guanylate cyclase activity are currently known: GC-A (or ANP-A) which seems specific to atrial natriuretic peptide (ANP), and GC-B (or ANP-B) which seems to be stimulated more effectively by brain natriuretic peptide (BNP) than by ANP.

-The receptor for Escherichia coli heat-stable enterotoxin (GC-C). The endogenous ligand for this intestinal receptor seems to be a small peptide called guanylin.

-Retinal guanylate cyclase (retGC) which probably plays a specific functional role in the rods and/or cones of photoreceptors. It is not known if this protein acts as receptor, but its structure is similar to that of the other plasma membrane-bound GCs.

The soluble forms of guanylate cyclase are cytoplasmic heterodimers. The two subunits, alpha and beta are proteins of from 70 to 82 Kd which are highly related. Two forms of beta subunits are currently known: beta-1 which seems to be expressed in lung and brain, and beta-2 which is more abundant in kidney and liver.

The membrane and cytoplasmic forms of guanylate cyclase share a conserved domain which is probably important for the catalytic activity of the enzyme. Such a domain is also found twice in the different forms of membrane-bound adenylate cyclases (also known as class-III) [5,6] from mammals, slime mold or Drosophila. A consensus pattern was derived from the most conserved region in that domain.

Consensus patternG-V-[LIVM]-x(0,1)-G-x(5)-[FY]-x-[LIVM]-[FYW]-[GS]-[DNTHKW]-[DNT]-[IV]-[DNTA]-x(5)-[DE]

Sequences known to belong to this class detected by the patternALL, except for the sea urchin Arbacia punctulata resact receptor which lack this domain.

Note this pattern will detect both domains of adenylate cyclases class-III.

[1]Koesling D., Boehme E., Schultz G. FASEB J. 5:2785-2791(1991).
[2]Garbers D.L. New Biol. 2:499-504(1990).
[3]Garbers D.L. Cell 71:1-4(1992).
[4]Yuen P.S.T., Garbers D.L. Annu. Rev. Neurosci. 15:193-225(1992).
[5]Iyengar R. FASEB J. 7:768-775(1993).
[6]Barzu O., Danchin A. Prog. Nucleic Acid Res. Mol. Biol. 49:241-283(1994).

956. Hemolysin-type calcium-binding region signature (HemolysinCabinD)

Gram-negative bacteria produce a number of proteins which are secreted into the growth medium by a mechanism that does not require a cleaved N-terminal signal sequence. These proteins, while having different functions, seem [1] to share two properties: they bind calcium and they contain a variable number of tandem repeats consisting of a nine amino acid motif rich in glycine, aspartic acid and asparagine. It has been shown [2] that such a domain is involved in the binding of calcium ions in a parallel beta roll structure. The proteins which are currently known to belong to this category are:

- Hemolysins from various species of bacteria. Bacterial hemolysins are exotoxins that attack blood cell membranes and cause cell rupture. The hemolysins which are known to contain such a domain are those from: E. coli (gene hlyA), A. pleuropneumoniae (gene appA), A. actinomycetemcomitans and P. haemolytica (leukotoxin) (gene lktA).
- Cyclolysin from Bordetella pertussis (gene cyaA). A multifunctional protein which is both an adenylate cyclase and a hemolysin.
- Extracellular zinc proteases: serralysin (EC 3.4.24.40) from Serratia, prtB and prtC from Erwinia chrysanthemi and aprA from Pseudomonas aeruginosa.
- Nodulation protein nodO from Rhizobium leguminosarum.

A signature pattern was derived from conserved positions in the sequence of the calcium-binding domain.

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Consensus pattern D-x-[LI]-x(4)-G-x-D-x-[LI]-x-G-G-x(3)-D Sequences known to belong to this class detected by the pattern ALL.

Note: This pattern is found once in nodO and the extracellular proteases but up to 5 times in some hemolysin/cyclolysins.

[1] Economou A., Hamilton W.D.O., Johnston A.W.B., Downie J.A. EMBO J. 9:349-354(1990).

[2] Baumann U., Wu S., Flaherty K.M., McKay D.B. EMBO J. 12:3357-3364(1993).

957. Hint module (Hint)

This is an alignment of the Hint module in the Hedgehog proteins. It does not include any Inteins which also possess the Hint module.

Number of members: 36

[1] Hall TM, Porter JA, Young KE, Koonin EV, Beachy PA, Leahy DJ; Medline: 97474313 "Crystal structure of a Hedgehog autoprocessing domain: homology between Hedgehog and self-splicing proteins." Cell 1997;91:85-97.

958. Hydantoinase/oxoprolinase (Hydantoinase)

This family includes the enzymes hydantoinase and oxoprolinase EC:3.5.2.9. Both reactions involve the hydrolysis of 5-membered rings via hydrolysis of their internal imide bonds [1].

Number of members: 14

09565355.051401

[1] Ye GJ, Breslow EB, Meister A, Guo-jie GE$[corrected to Ye GJ]; Medline: 97113037 "The amino acid sequence of rat kidney 5-oxo-L-prolinase determined by cDNA cloning" [published erratum appears in J Biol Chem 1997 Feb 14;272(7):4646] J Biol Chem 1996;271:32293-32300.

959. IMP dehydrogenase / GMP reductase signature (IMPDH_N)

IMP dehydrogenase (EC 1.1.1.205) (IMPDH) catalyzes the rate-limiting reaction of de novo GTP biosynthesis, the NAD-dependent reduction of IMP into XMP [1]. Inhibition of IMP dehydrogenase activity results in the cessation of DNA synthesis. As IMP dehydrogenase is associated with cell proliferation, it is a possible target for cancer chemotherapy. Mammalian and bacterial IMPDHs are tetramers of identical chains. There are two IMP dehydrogenase isozymes in humans [2].

GMP reductase (EC 1.6.6.8) catalyzes the irreversible and NADPH-dependent reductive deamination of GMP into IMP [3]. It converts nucleobase, nucleoside and nucleotide derivatives of G to A nucleotides, and maintains intracellular balance of A and G nucleotides.

IMP dehydrogenase and GMP reductase share many regions of sequence similarity. One of these regions is centered on a cysteine residue thought [3] to be involved in binding IMP. This region was used as a signature pattern.

Consensus pattern[LIVM]-[RK]-[LIVM]-G-[LIVM]-G-x-G-S-[LIVM]-C-x-T [C is the putative IMP-binding residue] Sequences known to belong to this class detected by the pattern ALL.

[1] Collart F.R., Huberman E. J. Biol. Chem. 263:15769-15772(1988).
[2] Natsumeda Y., Ohno S., Kawasaki H., Konno Y., Weber G., Suzuki K. J. Biol. Chem. 265:5292-5295(1990).
[3] Andrews S.C., Guest J.R. Biochem. J. 255:35-43(1988).

960. impB/mucB/samB family (IMS)

These proteins are involved in UV protection (Swiss).
Number of members: 38

961. Type II intron maturase (Intron_maturas2)

Group II introns use intron-encoded reverse transcriptase, maturase and DNA endonuclease activities for site-specific insertion into DNA [2]. Although this type of intron is self splicing in vitro they require a maturase protein for splicing in vivo. It has been shown that a specific region of the aI2 intron is needed for the maturase function [1]. This region was found to be conserved in group II introns and called domain X [3].
Number of members: 335

[1] Moran JV, Mecklenburg KL, Sass P, Belcher SM, Mahnke D, Lewin A, Perlman P; Medline: 94301788 “Splicing defective mutants of the COXI gene of yeast mitochondrial DNA: initial definition of the maturase domain of the group II intron aI2. Nucleic Acids Res 1994;22:2057-2064.

[2] Guo H, Zimmerly S, Perlman PS, Lambowitz AM; Medline: 98031910 “Group II intron endonucleases use both RNA and protein subunits for recognition of specific sequences in double-stranded DNA.” EMBO J 1997;16:6835-6848.

[3] Mohr G, Perlman PS, Lambowitz AM; Medline: 94077696 “Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function.” Nucleic Acids Res 1993;21:4991-4997.

962. LAGLIDADG endonuclease (Intron_maturase)

[1] Heath PJ, Stephens KM, Monnat RJ Jr, Stoddard BL; Medline: 97331323 “The structure of I-CreI, a group I intron-encoded homing endonuclease.” Nat Struct Biol 1997;4:468-476.

[2] Belfort M, Roberts RJ; Medline: 97402526 “Homing endonucleases: keeping the house in order.” Nucleic Acids Res 1997;25:3379-3388.

[3] Dalgaard JZ, Klar AJ, Moser MJ, Holley WR, Chatterjee A, Mian IS; Medline: 98026854 “Statistical modeling and analysis of the LAGLIDADG family of site-specific endonucleases and identification of an intein that encodes a site-specific endonuclease of the HNH family.” Nucleic Acids Res 1997;25:4626-4638.

Number of members: 220

963. Isopentenyl transferase (IPT)

Isopentenyl transferase / dimethylallyl transferase synthesizes isopentenyladensosine 5'-monophosphate, a cytokinin that induces shoot formation on host plants infected with the Ti plasmid [1].

Number of members: 16

[1] Canaday J, Gerad JC, Crouzet P, Otten L; Medline: 93101133 "Organization and functional analysis of three T-DNAs from the vitopine Ti plasmid pTiS4." Mol Gen Genet 1992;235:292-303.

964. Laminin EGF-like (Domains III and V) (laminin_EGF)

This family is like EGF but has 8 conserved cysteines instead of 6.

Number of members: 501

[1] Engel J; Medline: 93041759 "Laminins and other strange proteins." Biochemistry 1992;31:10643-10651.

965. Legume lectins signatures (lectin_legA)

Leguminous plants synthesize sugar-binding proteins which are called legume lectins [1,2]. These lectins are generally found in the seeds. The exact function of legume lectins is not known but they may be involved in the attachment of nitrogen-fixing bacteria to legumes and in the protection against pathogens. Legume lectins bind calcium and manganese (or other transition metals).

Legume lectins are synthesized as precursor proteins of about 230 to 260 amino acid residues. Some legume lectins are proteolytically processed to produce two chains: beta (which corresponds to the N-terminal) and alpha (C-terminal). The lectin concanavalin A (conA) from jack bean is exceptional in that the two chains are transposed and ligated (by formation of a new peptide bond). The N-terminus of mature conA thus corresponds to that of the alpha chain and the C-terminus to the beta chain.

Two signature patterns were developed specific to legume lectins: the first is located in the C-terminal section of the beta chain and contains a conserved aspartic acid residue important for the binding of calcium and manganese; the second one is located in the N-terminal of the alpha chain.

Consensus pattern [LIV]-[STAG]-V-[DEQV]-[FLI]-D-[ST] [D binds manganese and calcium] Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [LIV]-x-[EDQ]-[FYWKR]-V-x-[LIVF]-G-[LF]-[ST] Sequences known to belong to this class detected by the pattern ALL.

[1] Sharon N., Lis H. FASEB J. 4:3198-320(1990).

[2] Lis H., Sharon N. Annu. Rev. Biochem. 55:33-37(1986).

966. Malate synthase signature (malate_synthase)

Malate synthase (EC 4.1.3.2) catalyzes the aldol condensation of glyoxylate with acetyl-CoA to form malate - the second step of the glyoxylate bypass, an alternative to the tricarboxylic acid cycle in bacteria, fungi and plants. Malate synthase is a protein of 530 to 570 amino acids whose sequence is highly conserved across species [1]. As a signature pattern, a very conserved region was selected in the central section of the enzyme.

Consensus pattern[KR]-[DENQ]-H-x(2)-G-L-N-x-G-x-W-D-Y-[LIVM]-F Sequences known to belong to this class detected by the pattern ALL.

[1] Bruinenberg P.G., Blaauw M., Kazemier B., Ab G. Yeast 6:245-254(1990).

967. MatK/TrnK amino terminal region (MatK_N)

[1] Mohr G, Perlman PS, Lambowitz AM; Medline: 94077696 “Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function.” Nucleic Acids Res 1993;21:4991-4997.

Number of members: 495

968. MOZ/SAS family (MOZ_SAS)

This region of these proteins has been suggested to be homologous to acetyltransferases [1]. However the similarity is not supported by standard sequence analysis.
Number of members: 15

[1] Kamine J, Elangovan B, Subramanian T, Coleman D, Chinnadurai G; Medline: 96182937 “Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator.” Virology 1996;216:357-366.

[2] Reifsnyder C, Lowell J, Clarke A, Pillus L; Medline: 96376969 "Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases" [see comments] [published erratum appears in Nat Genet 1997 May;16(1):109] Nat Genet 1996;14:42-49.

969. mRNA capping enzyme (mRNA_cap_enzyme)

[1] Hakansson K, Doherty AJ, Shuman S, Wigley DB; Medline: 97304383 "X-ray crystallography reveals a large conformational change during guanyl transfer by mRNA capping enzymes." Cell 1997;89:545-553.

Number of members: 7

970. DNA mismatch repair proteins mutS family signature (MutS_C)

Mismatch repair contributes to the overall fidelity of DNA replication [1]. It involves the correction of mismatched base pairs that have been missed by the proofreading element of the DNA polymerase complex. The sequence of some proteins involved in mismatch repair in different organisms have been found to be evolutionary related [2,3]. One of these families is called mutS [4,E1], it consists of:

- Prokaroytic protein mutS protein (also called hexA in Streptococcus pneumoniae). Muts is thought to carry out the mismatch recognition step of DNA repair.
- Eukaryotic MSH1, which is involved in mitochondrial DNA repair.
- Eukaryotic MSH2, which is involved in nuclear postreplication mismatch repair. MSH2 heterodimerizes with MSH6. In man, MSH2 is involved in a form of familial hereditary nonpolyposis colon cancer (HNPCC).
- Eukaryotic MSH3, which is probably involved in the repair of large loops.
- Eukaryotic MSH4, which is involved in meiotic recombination.
- Eukaryotic MSH5, which is involved in meiotic recombination.
- Eukaryotic MSH6 (also known as G/T mismatch binding protein), a DNA-repair protein that binds to G/T mismatches through heterodimerization with MSH2.
- Prokaryotic protein mutS2 whose function is not yet known.
- A coral (Sarcophyton glaucum) mitochondrial encoded mutS-like protein.

As a signature pattern for this class of mismatch repair proteins a region rich in glycine and negatively charged residues was selected This region is found in the C-terminal section of these proteins; about 80 residues to the C-terminal of an ATP-binding site motif 'A' (P-loop) (see <PDOC00017>).

Consensus pattern[ST]-[LIVMF]-x-[LIVM]-x-D-E-[LIVMFY]-[GC]-[RKH]-G-[GST]- x(4)-G Sequences known to belong to this class detected by the pattern ALL, except for mutS2.

[1] Modrich P. Annu. Rev. Biochem. 56:435-466(1987).
[2] Haber L.T., Walker G.C. EMBO J. 10:2707-2715(1991).
[3] New L., Liu K., Crouse G.F. Mol. Gen. Genet. 239:97-108(1993).
[4] Eisen J.A. Nucleic Acids Res. 26:4291-4300(1998).

971. MutS family, N-terminal putative DNA binding domain (MutS_N)

This family consists of the N-terminal region of proteins in the mutS family of DNA mismatch repair proteins and is found associated with MutS_C located in the C-terminal region. The mutS family of proteins is named after the salmonella typhimurium MutS protein involved in mismatch repair; other members of the family included the eukaryotic MSH 1,2,3,4,5 and 6 proteins. These have various roles in DNA repair and recombination. Human MSH has been implicated in non-polyposis colorectal carcinoma (HNPCC) and is a mismatch binding protein [2]. The aligned region corresponds in part with domains A1, A2 (which may bind DNA) and B (which binds dsDNA in vitro) from T. thermophilus MutS as characterised in [1].

Number of members: 43

972. Domain in Myosin and Kinesin Tails (MyTH4)

Domain present twice in myosin-VIIa, and also present in 3 other myosins.

[1] Chen ZY, Hasson T, Kelley PM, Schwender BJ, Schwartz MF, Ramakrishnan M, Kimberling WJ, Mooseker MS, Corey DP; Medline: 97038686 “Molecular cloning and

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domain structure of human myosin-VIIa, the gene product defective in Usher syndrome 1B." Genomics 1996;36:440-448.

Number of members: 21

973. Sodium and potassium ATPases beta subunits signatures (Na_K-ATPase)

The sodium pump (Na+,K+ ATPase), located in the plasma membrane of all animal cells [1], is an heterotrimer of a catalytic subunit (alpha chain), a glycoprotein subunit of about 34 Kd (beta chain) and a small hydrophobic protein of about 6 Kd. The beta subunit seems [2] to regulate, through the assembly of alpha/beta heterodimers, the number of sodium pumps transported to the plasma membrane.

Structurally the beta subunit is composed of a charged cytoplasmic domain of about 35 residues, followed by a transmembrane region, and a large extracellular domain that contains three disulfide bonds and glycosylation sites. This structure is schematically represented in the figure below.

```
 +----+ +--+ +-----------+ ||||||
xxxxxxxxxxxxxxxxxxxxxxxxxCxxxxCxCxxCxxxxxxxCxxxxxxxxxxxCxxxx
 **** **** <-Cyt-><TM><------------Extracellular-------------------->
```

'C': conserved cysteine involved in a disulfide bond.
'*': position of the patterns.

Two isoforms of the beta subunit (beta-1 and beta-2) are currently known; they share about 50% sequence identity. Gastric (K+, H+) ATPase (proton pump) responsible for acid production in the stomach consist of two subunits [3]; the beta chain is highly similar to the sodium pump beta subunits. Two signature patterns were developed for beta subunits. The first is located in the cytoplasmic domain, while the second is found in the extracellular domain and contains two of the cysteines involved in disulfide bonds.

Consensus pattern [FYW]-x(2)-[FYW]-x-[FYW]-[DN]-x(6)-[LIVM]-G-R-T-x(3)-W
Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [RK]-x(2)-C-[RKQWI]-x(5)-L-x(2)-C-[SA]-G [The two C's are involved in disulfide bonds] Sequences known to belong to this class detected by the patternALL, except for the beta subunit of the sodium pump of brine shrimp whose sequence is highly divergent in that region.

[1] Horisberger J.D., Lemas V., Krahenbul J.P., Rossier B.C. Annu. Rev. Physiol. 53:565-584(1991).
[2] McDonough A.A., Gerring K., Farley R.A. FASEB J. 4:1598-1605(1990).
[3] Toh B.-H., Gleeson P.A., Simpson R.J., Moritz R.L., Callaghan J.M., Goldkorn I., Jones C.M., Martinelli T.M., Mu F.-T., Humphris D.C., Pettitt J.M., Mori Y., Masuda T., Sobieszczuk P., Weinstock J., Mantamadiotis T., Baldwin G.S. Proc. Natl. Acad. Sci. U.S.A. 87:6418-6422(1990).

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974. Respiratory-chain NADH dehydrogenase subunit 1 signatures (NADHdh)

Respiratory-chain NADH dehydrogenase (EC 1.6.5.3) [1,2] (also known as complex I or NADH-ubiquinone oxidoreductase) is an oligomeric enzymatic complex located in the inner mitochondrial membrane which also seems to exist in the chloroplast and in cyanobacteria (as a NADH-plastoquinone oxidoreductase). Among the 25 to 30 polypeptide subunits of this bioenergetic enzyme complex there are fifteen which are located in the membrane part, seven of which are encoded by the mitochondrial and chloroplast genomes of most species. The most conserved of these organelle-encoded subunits is known as subunit 1 (gene ND1 in mitochondrion, and NDH1 in chloroplast) and seems to contain the ubiquinone binding site.

The ND1 subunit is highly similar to subunit 4 of Escherichia coli formate hydrogenlyase (gene hycD), subunit C of hydrogenase-4 (gene hyfC). Paracoccus denitrificans NQO8 and Escherichia coli nuoH NADH-ubiquinone oxidoreductase subunits also belong to this family [3]. Two signature patterns were developed based on conserved regions of this subunit.

Consensus pattern G-[LIVMFYKRS]-[LIVMAGP]-Q-x-[LIVMFY]-x-D-[AGIM]-[LIVMFTA]- K-[LVMYST]-[LIVMFYG]-x-[KR]-[EQG] Sequences known to belong to this class detected by the patternALL, except for watermelon and Leishmania ND1.

Consensus pattern P-F-D-[LIVMFYQ]-[STAGPVM]-E-[GAC]-E-x-[EQ]-[LIVMS]-x(2)-G Sequences known to belong to this class detected by the pattern ALL, except for Chlamydomonas reinhardtii and Pisaster ochraceus ND1, and tobacco NDH1.

[1] Ragan C.I. Curr. Top. Bioenerg. 15:1-36(1987).
[2] Weiss H., Friedrich T., Hofhaus G., Preis D. Eur. J. Biochem. 197:563-576(1991).
[3] Weidner U., Geier S., Ptock A., Friedrich T., Leif H., Weiss H. J. Mol. Biol. 233:109-122(1993).

975. Nickel-dependent hydrogenases large subunit signatures (NiFeSe_Hases)

Hydrogenases are enzymes that catalyze the reversible activation of hydrogen and which occur widely in prokaryotes as well as in some eukaryotes. There are various types of hydrogenases, but all of them seem to contain at least one iron-sulfur cluster. They can be broadly divided into two groups: hydrogenases containing nickel and, in some cases, also selenium (the [NiFe] and [NiFeSe] hydrogenases) and those lacking nickel (the [Fe] hydrogenases).

The [NiFe] and [NiFeSe] hydrogenases are heterodimer that consist of a small subunit that contains a signal peptide and a large subunit. All the known large subunits seem to be evolutionary related [1]; they contain two Cys-x-x- Cys motifs; one at their N-terminal end; the other at their C-terminal end. These four cysteines are involved in the binding of nickel [2]. In the [NiFeSe] hydrogenases the first cysteine of the C-terminal motif is a selenocysteine which has experimentally been shown to be a nickel ligand [3]. Two patterns were developed which are centered on the Cys-x-x-Cys motifs.

Alcaligenes eutrophus possess a NAD-reducing cytoplasmic hydrogenase (hoxS) [4]; this enzyme is composed of four subunits. Two of these subunits (beta and delta) are responsible for the hydrogenase reaction and are evolutionary related to the large and small subunits of membrane-bound hydrogenases. The alpha subunit of coenzyme F420 hydrogenase (EC 1.12.99.1) (FRH) from archaebacterial methanogens also belongs to this family.

Consensus pattern R-G-[LIVMF]-E-x(15)-[QESM]-R-x-C-G-[LIVM]-C [The two C's are nickel ligands] Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern [FY]-D-P-C-[LIM]-[ASG]-C-x(2,3)-H [The two C's are nickel ligands] Sequences known to belong to this class detected by the pattern ALL.

[1] Menon N.K., Robbins J., Peck H.D. Jr., Chatelus C.Y., Choi E.-S., Przybyla A.E. J. Bacteriol. 172:1969-1977(1990).
[2] Volbeda A., Charon M.-H., Piras C., Hatchikian E.C., Frey M., Fontecilla-Camps J.C. Nature 373:580-587(1995).
[3] Eidsness M.K., Scott R.A., Prickrill B., der Vartaninan D.V., LeGall J., Moura I., Moura J.J.G., Peck H.D. Jr. Proc. Natl. Acad. Sci. U.S.A. 86:147-151(1989).
[4] Tran-Betcke A., Warnecke U., Boecker C., Zaborosch C., Friedrich B. J. Bacteriol. 172:2920-2929(1990).

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976. NADH-Ubiquinone oxidoreductase (complex I), chain 5 C-terminus (oxidored_q1_C)

This sub-family represents a carboxyl terminal extension of oxidored_q1. Only NADH-Ubiquinone chain 5 from chloroplasts are in this family. This sub-family is part of complex I which catalyses the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane.
Number of members: 572

[1] Walker JE; Medline: 93110040 “The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains.” Q Rev Biophys 1992;25:253-324.

977. NADH-Ubiquinone oxidoreductase (complex I), chain 5 N-terminus (oxidored_q1_N)

This sub-family represents an amino terminal extension of oxidored_q1. Only NADH-Ubiquinone chain 5 and eubacterial chain L are in this family. This sub-family is part of complex I which catalyses the transfer of two electrons from NADH to ubiquinone in a reaction that is associated with proton translocation across the membrane.
Number of members: 546

[1] Walker JE; Medline: 93110040 “The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains.” Q Rev Biophys 1992;25:253-324.

978. oxidored_q2. NADH-UBIQUINONE OXIDOREDUCTASE CHAIN 4L (EC 1.6.5.3). ND4L OR NAD4L. Arabidopsis thaliana (Mouse-ear cress). Mitochondrion. OC Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; Rosidae; eurosids II; Brassicales; Brassicaceae; Arabidopsis.
CATALYTIC ACTIVITY: NADH + UBIQUINONE = NAD(+) + UBIQUINOL.

[1] SEQUENCE FROM N.A. MEDLINE; 93156682. Brandt P., Sunkel S., Unseld M., Brennicke A., Knoop V.; "The nad4L gene is encoded between exon c of nad5 and orf25 in the Arabidopsis mitochondrial genome."; Mol. Gen. Genet. 236:33-38(1992).
[2] SEQUENCE FROM N.A. STRAIN=CV. COLUMBIA; MEDLINE; 97141919 Unseld M., Marienfeld J.R., Brandt P., Brennicke A.; "The mitochondrial genome of Arabidopsis thaliana contains 57 genes in 366,924 nucleotides."; Nat. Genet. 15:57-61(1997).

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979. oxidored_q4. Protein name NADH-PLASTOQUINONE OXIDOREDUCTASE CHAIN 3, CHLOROPLAST. Synonym(s)EC 1.6.5.3. Gene name(s)NDHC OR NDH3 From Zea mays (Maize) Encoded on Chloroplast. Taxonomy Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Zea.

CATALYTIC ACTIVITY: NADH + PLASTOQUINONE = NAD(+) + PLASTOQUINOL.

SIMILARITY: BELONGS TO THE COMPLEX I SUBUNIT 3 FAMILY.

[1] SEQUENCE FROM N.A. MEDLINE; 89281491. Steinmueller K., Ley A.C., Steinmetz A.A., Sayre R.T., Bogorad L.; "Characterization of the ndhC-psbG-ORF157/159 operon of maize plastid DNA and of the cyanobacterium Synechocystis sp. PCC6803."; Mol. Gen. Genet. 216:60-69(1989).
[2] SEQUENCE FROM N.A. MEDLINE; 95395841. Maier R.M., Neckermann K., Igloi G.L., Koessel H.; "Complete sequence of the maize chloroplast genome: gene content, hotspots of divergence and fine tuning of genetic information by transcript editing."; J. Mol. Biol. 251:614-628(1995).

980. PAC: PAC motif

PAC motif occurs C-terminal to a subset of all known PAS motifs. It is proposed to contribute to the PAS domain fold [3]. Number of members: 181

[1] Medline: 97446881 PAS domain S-boxes in archaea, bacteria and sensors for oxygen and redox. Zhulin IB, Taylor BL, Dixon R; Trends Biochem Sci 1997;22:331-333.

[2] Medline: 95275818. 1.4 A structure of photoactive yellow protein, a cytosolic photoreceptor: unusual fold, active site, and chromophore. Borgstahl GE, Williams DR, Getzoff ED; Biochemistry 1995;34:6278-6287.

[3] Medline: 98044337. PAS: a multifunctional domain family comes to light. Ponting CP, Aravind L; Curr Biol 1997;7:674-677.

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981. PARP: Poly(ADP-ribose) polymerase catalytic region.

Poly(ADP-ribose) polymerase catalyses the covalent attachment of ADP-ribose units from NAD+ to itself and to a limited number of other DNA binding proteins, which decreases their affinity for DNA. Poly(ADP-ribose) polymerase is a regulatory component induced by DNA damage.

The carboxyl-terminal region is the most highly conserved region of the protein. Experiments have shown that a carboxyl 40 kDa fragment is still catalytically active [2]. Number of members: 19

[1] Medline: 96353841 Structure of the catalytic fragment of poly(AD-ribose) polymerase from chicken. Ruf A, Mennissier de Murcia J, de Murcia G, Schulz GE; Proc Natl Acad Sci U S A 1996;93:7481-7485.

[2] Medline: 93293867 The carboxyl-terminal domain of human poly(ADP-ribose) polymerase. Overproduction in Escherichia coli, large scale purification, and characterization. Simonin F, Hofferer L, Panzeter PL, Muller S, de Murcia G, Althaus FR; J Biol Chem 1993;268:13454-13461.

982. PC_rep: Proteasome/cyclosome repeat

[1] Medline: 97348748 A repetitive sequence in subunits of the 26S proteasome and 20S cyclosome (anaphase-promoting complex). Lupas A, Baumeister W, Hofmann K; Trends Biochem Sci 1997;22:195-196.

Number of members: 112

983. Peptidase_M1: Peptidase family M1

Members of this family are aminopeptidases. The members differ widely in specificity, hydrolysing acidic, basic or neutral N-terminal residues. This family includes leukotriene-A4 hydrolase Swiss:P09960, this enzyme also has an aminopeptidase activity [1]. Number of members: 72

[1] Medline: 95405261 Evolutionary families of metallopeptidases. Rawlings ND, Barrett AJ; Meth Enzymol 1995;248:183-228.

09525355.051601

984. Neutral zinc metallopeptidases, zinc-binding region signature (Peptidase_M8)

PROSITE cross-reference(s) PS00142; ZINC_PROTEASE

The majority of zinc-dependent metallopeptidases (with the notable exception of the carboxypeptidases) share a common pattern of primary structure [1,2,3] in the part of their sequence involved in the binding of zinc, and can be grouped together as a superfamily,known as the metzincins, on the basis of this sequence similarity. They can be classified into a number of distinct families [4,E1] which are listed below along with the proteases which are currently known to belong to these families.

Family M1

- Bacterial aminopeptidase N (EC 3.4.11.2) (gene pepN).
- Mammalian aminopeptidase N (EC 3.4.11.2).
- Mammalian glutamyl aminopeptidase (EC 3.4.11.7) (aminopeptidase A). It may play a role in regulating growth and differentiation of early B-lineage cells.
- Yeast aminopeptidase yscII (gene APE2).
- Yeast alanine/arginine aminopeptidase (gene AAP1).
- Yeast hypothetical protein YIL137c.

- Leukotriene A-4 hydrolase (EC 3.3.2.6). This enzyme is responsible for the hydrolysis of an epoxide moiety of LTA-4 to form LTB-4; it has been shown that it binds zinc and is capable of peptidase activity.

Family M2

- Angiotensin-converting enzyme (EC 3.4.15.1) (dipeptidyl carboxypeptidase I) (ACE) the enzyme responsible for hydrolyzing angiotensin I to angiotensin II. There are two forms of ACE: a testis-specific isozyme and a somatic isozyme which has two active centers.

Family M3

- Thimet oligopeptidase (EC 3.4.24.15), a mammalian enzyme involved in the cytoplasmic degradation of small peptides.
- Neurolysin (EC 3.4.24.16) (also known as mitochondrial oligopeptidase M or microsomal endopeptidase).
- Mitochondrial intermediate peptidase precursor (EC 3.4.24.59) (MIP). It is involved the second stage of processing of some proteins imported in the mitochondrion.
- Yeast saccharolysin (EC 3.4.24.37) (proteinase yscD).
- Escherichia coli and related bacteria dipeptidyl carboxypeptidase (EC 3.4.15.5) (gene dcp).
- Escherichia coli and related bacteria oligopeptidase A (EC 3.4.24.70) (gene opdA or prlC).
- Yeast hypothetical protein YKL134c.

Family M4

- Thermostable thermolysins (EC 3.4.24.27), and related thermolabile neutral proteases (bacillolysins) (EC 3.4.24.28) from various species of Bacillus.
- Pseudolysin (EC 3.4.24.26) from Pseudomonas aeruginosa (gene lasB).
- Extracellular elastase from Staphylococcus epidermidis.
- Extracellular protease prt1 from Erwinia carotovora.
- Extracellular minor protease smp from Serratia marcescens.
- Vibriolysin (EC 3.4.24.25) from various species of Vibrio.
- Protease prtA from Listeria monocytogenes.
- Extracellular proteinase proA from Legionella pneumophila.

Family M5

- Mycolysin (EC 3.4.24.31) from Streptomyces cacaoi.

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Family M6

- Immune inhibitor A from Bacillus thuringiensis (gene ina). Ina degrades two classes of insect antibacterial proteins, attacins and cecropins.

Family M7

- Streptomyces extracellular small neutral proteases

Family M8

- Leishmanolysin (EC 3.4.24.36) (surface glycoprotein gp63), a cell surface protease from various species of Leishmania.

Family M9

- Microbial collagenase (EC 3.4.24.3) from Clostridium perfringens and Vibrio alginolyticus.

Family M10A

- Serralysin (EC 3.4.24.40), an extracellular metalloprotease from Serratia.
- Alkaline metalloproteinase from Pseudomonas aeruginosa (gene aprA).
- Secreted proteases A, B, C and G from Erwinia chrysanthemi.
- Yeast hypothetical protein YIL108w.

Family M10B

- Mammalian extracellular matrix metalloproteinases (known as matrixins) [5]: MMP-1 (EC 3.4.24.7) (interstitial collagenase), MMP-2 (EC 3.4.24.24) (72 Kd gelatinase), MMP-9 (EC 3.4.24.35) (92 Kd gelatinase), MMP-7 (EC 3.4.24.23) (matrylisin), MMP-8 (EC 3.4.24.34) (neutrophil collagenase), MMP-3 (EC 3.4.24.17) (stromelysin-1), MMP-10 (EC 3.4.24.22) (stromelysin-2), and MMP-11 (stromelysin-3), MMP-12 (EC 3.4.24.65) (macrophage metalloelastase).
- Sea urchin hatching enzyme (envelysin) (EC 3.4.24.12). A proteas that allows the embryo to digest the protective envelope derived from the egg extracellular matrix.
- Soybean metalloendoproteinase 1.

Family M11

- Chlamydomonas reinhardtii gamete lytic enzyme (GLE).

Family M12A

- Astacin (EC 3.4.24.21), a crayfish endoprotease.
- Meprin A (EC 3.4.24.18), a mammalian kidney and intestinal brush border metalloendopeptidase.
- Bone morphogenic protein 1 (BMP-1), a protein which induces cartilage and bone formation and which expresses metalloendopeptidase activity. The Drosophila homolog of BMP-1 is the dorsal-ventral patterning protein tolloid.
- Blastula protease 10 (BP10) from Paracentrotus lividus and the related protein SpAN from Strongylocentrotus purpuratus.
- Caenorhabditis elegans protein toh-2.
- Caenorhabditis elegans hypothetical protein F42A10.8.
- Choriolysins L and H (EC 3.4.24.67) (also known as embryonic hatching proteins LCE and HCE) from the fish Oryzias lapides. These proteases participates in the breakdown of the egg envelope, which is derived from the egg extracellular matrix, at the time of hatching.

Family M12B

- Snake venom metalloproteinases [6]. This subfamily mostly groups proteases that act in hemorrhage. Examples are: adamalysin II (EC 3.4.24.46), atrolysin C/D (EC 3.4.24.42), atrolysin E (EC 3.4.24.44), fibrolase (EC 3.4.24.72), trimerelysin I (EC 3.4.25.52) and II (EC 3.4.25.53).
- Mouse cell surface antigen MS2.

Family M13

- Mammalian neprilysin (EC 3.4.24.11) (neutral endopeptidase) (NEP).
- Endothelin-converting enzyme 1 (EC 3.4.24.71) (ECE-1), which process the precursor of endothelin to release the active peptide.
- Kell blood group glycoprotein, a major antigenic protein of erythrocytes. The Kell protein is very probably a zinc endopeptidase.
- Peptidase O from Lactococcus lactis (gene pepO).

09955363.091601

Family M27

- Clostridial neurotoxins, including tetanus toxin (TeTx) and the various botulinum toxins (BoNT). These toxins are zinc proteases that block neurotransmitter release by proteolytic cleavage of synaptic proteins such as synaptobrevins, syntaxin and SNAP-25 [7,8].

Family M30

- Staphylococcus hyicus neutral metalloprotease.

Family M32

- Thermostable carboxypeptidase 1 (EC 3.4.17.19) (carboxypeptidase Taq), an enzyme from Thermus aquaticus which is most active at high temperature.

Family M34

- Lethal factor (LF) from Bacillus anthracis, one of the three proteins composing the anthrax toxin.

Family M35

- Deuterolysin (EC 3.4.24.39) from Penicillium citrinum and related proteases from various species of Aspergillus.

Family M36

- Extracellular elastinolytic metalloproteinases from Aspergillus.

From the tertiary structure of thermolysin, the position of the residues acting as zinc ligands and those involved in the catalytic activity are known. Two of the zinc ligands are histidines which are very close together in the sequence; C-terminal to the first histidine is a glutamic acid residue which acts as a nucleophile and promotes the attack of a water molecule on the carbonyl carbon of the substrate. A signature pattern which includes the two histidine and the glutamic acid residues is sufficient to detect this superfamily of proteins.

Consensus pattern[GSTALIVN]-x(2)-H-E-[LIVMFYW]-{DEHRKP}-H-x-[LIVMFYWGSPQ]

[The two H's are zinc ligands] [E is the active site residue]

Sequences known to belong to this class detected by the patternALL, except for members of families M5, M7 amd M11.

Other sequence(s) detected in SWISS-PROT57; including Neurospora crassa conidiation-specific protein 13 which could be a zinc-protease.

[1]Jongeneel C.V., Bouvier J., Bairoch A. FEBS Lett. 242:211-214(1989).

[2]Murphy G.J.P., Murphy G., Reynolds J.J. FEBS Lett. 289:4-7(1991).

[3]Bode W., Grams F., Reinemer P., Gomis-Rueth F.-X., Baumann U., McKay D.B., Stoecker W. Zoology 99:237-246(1996).

[4]Rawlings N.D., Barrett A.J. Meth. Enzymol. 248:183-228(1995).

[5]Woessner J. Jr. FASEB J. 5:2145-2154(1991).

[6]Hite L.A., Fox J.W., Bjarnason J.B. Biol. Chem. Hoppe-Seyler 373:381-385(1992).

[7]Montecucco C., Schiavo G. Trends Biochem. Sci. 18:324-327(1993).

[8]Niemann H., Blasi J., Jahn R. Trends Cell Biol. 4:179-185(1994).

985. PHO4: Phosphate transporter family

This family includes PHO-4 from Neurospora crassa which is a is a Na(+)-phosphate symporter [1]. This family also contains the leukemia virus receptor Swiss:Q08344. Number of members: 41

[1] Medline: 95249577 Repressible cation-phosphate symporters in Neurospora crassa. Versaw WK, Metzenberg RL; Proc Natl Acad Sci U S A 1995;92:3884-3887.

986. Photosynthetic reaction center proteins signature (photoRC)

PROSITE cross-reference(s): PS00244; REACTION_CENTER

In the photosynthetic reaction center of purple bacteria, two homologous integral membrane proteins, L(ight) and M(edium), are known to be essential to the light-mediated water-splitting process. In the photosystem II of eukaryotic chloroplasts two related proteins are involved: the D1 (psbA) and D2 proteins (psbD). These four types of protein probably evolved from a common ancestor [see 1,2 for recent reviews].

A signature pattern was developed which include two conserved histidine residues. In L and M chains, the first histidine is a ligand of the magnesium ion of the special pair bacteriochlorophyll, the second is a ligand of a ferrous non-heme iron atom. In photosystem II these two histidines are thought to play a similar role.

Consensus pattern[NQH]-x(4)-P-x-H-x(2)-[SAG]-x(11)-[SAGC]-x-H-[SAG](2)
[The first H is a magnesium ligand] [The second H is a iron ligand]
Sequences known to belong to this class detected by the patternALL, except for broad bean psbA which has Gln instead of the second His.

[1]Michel H., Deisenhofer J. Biochemistry 27:1-7(1988).
[2]Barber J. Trends Biochem. Sci. 12:321-326(1987).

987. phytochrome: Phytochrome region
This family contains a region specific to phytochrome proteins. Number of members: 145

988. PI3K_C2: C2 domain
Phosphoinositide 3-kinase region postulated to contain a C2 domain. Outlier of C2 family.
Number of members: 39

[1] Medline: 97388296 Using structure to define the function of phosphoinositide 3-kinase family members. Domin J, Waterfield MD; FEBS Lett 1997;410:91-95.
[2] Medline: 97398940 Phosphoinositide 3-kinases: a conserved family of signal transducers. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD; Trends Biochem Sci 1997;22:267-272.

989. PI3Ka: Phosphoinositide 3-kinase family, accessory domain (PIK domain)
PIK domain is conserved in all PI3 and PI4-kinases. Its role is unclear but it has been suggested [2] to be involved in substrate presentation.
Number of members: 47

[1] Medline: 97388296 Using structure to define the function of phosphoinositide 3-kinase family members. Domin J, Waterfield MD; FEBS Lett 1997;410:91-95.
[2] Medline: 94069320 Phosphatidylinositol 4-kinase: gene structure and requirement for yeast cell viability. Flanagan CA, Schnieders EA, Emerick AW, Kunisawa R, Admon A, Thorner J; Science 1993;262:1444-1448.

990. P-II protein signatures

PROSITE cross-reference(s): PS00496; PII_GLNB_UMP, PS00638; PII_GLNB_CTER

The P-II protein (gene glnB) is a bacterial protein important for the control of glutamine synthetase [1,2,3]. In nitrogen-limiting conditions, when the ratio of glutamine to 2-ketoglutarate decreases, P-II is uridylylated on a tyrosine residue to form P-II-UMP. P-II-UMP allows the deadenylation of glutamine synthetase (GS), thus activating the enzyme. Conversely, in nitrogen excess, P-II-UMP is deuridylated and then promotes the adenylation of GS. P-II also indirectly controls the transcription of the GS gene (glnA) by preventing NR-II (ntrB) to phosphorylate NR-I (ntrC) which is the transcriptional activator of glnA. Once P-II is uridylylated, these events are reversed.

P-II is a protein of about 110 amino acid residues extremely well conserved. The tyrosine which is urydylated is located in the central part of the protein.

In cyanobacteria, P-II seems to be phosphorylated on a serine residue rather than being urydylated.

In methanogenic archaebacteria, the nitrogenase iron protein gene (nifH) is followed by two open reading frames highly similar to the eubacterial P-II protein [4]. These proteins could be involved in the regulation of nitrogen fixation.

In the red alga, Porphyra purpurea, there is a glnB homolog encoded in the chloroplast genome.

Other proteins highly similar to glnB are:

- Bacillus subtilis protein nrgB [5].
- Escherichia coli hypothetical protein ybaI [6].

Two signature patterns were developed for P-II protein. The first one is a conserved stretch (in eubacteria) of six residues which contains the urydylated tyrosine, the other is derived from a conserved region in the C-terminal part of the P-II protein.

Consensus patternY-[KR]-G-[AS]-[AE]-Y [The second Y is uridylated]
Sequences known to belong to this class detected by the patternALL glnB's from eubacteria.
Consensus pattern[ST]-x(3)-G-[DY]-G-[KR]-[IV]-[FW]-[LIVM]-x(2)-[LIVM]

[1]Magasanik B. Biochimie 71:1005-1012(1989).
[2]Holtel A., Merrick M. Mol. Gen. Genet. 215:134-138(1988).
[3]Cheah E., Carr P.D., Suffolk P.M., Vasuvedan S.G., Dixon N.E., Ollis D.L. Structure 2:981-990(1994).
[4]Sibold L., Henriquet M., Possot O., Aubert J.-P. Res. Microbiol. 142:5-12(1991).
[5]Wray L.V. Jr., Atkinson M.R., Fisher S.H. J. Bacteriol. 176:108-114(1994).
[6]Allikmets R., Gerrard B.C., Court D., Dean M.C. Gene 136:231-236(1993).

991. PIP5K: Phosphatidylinositol-4-phosphate 5-Kinase

This family contains a region from the common kinase core found in the type I phosphatidylinositol-4-phosphate 5-kinase (PIP5K) family as described in [1]. The family consists of various type I, II and III PIP5K enzymes. PIP5K catalyses the formation of phosphoinositol-4,5-bisphosphate via the phosphorylation of phosphatidylinositol-4-phosphate a precursor in the phosphinositide signaling pathway. Number of members: 33

[1] Medline: 98204859. Type I phosphatidylinositol-4-phosphate 5-kinases. Cloning of the third isoform and deletion/substitution analysis of members of this novel lipid kinase family. Ishihara H, Shibasaki Y, Kizuki N, Wada T, Yazaki Y, Asano T, Oka Y; J Biol Chem 1998;273:8741-8748.

[2] Medline: 97115834 Type I phosphatidylinositol-4-phosphate 5-kinases are distinct members of this novel lipid kinase family. Loijens JC, Anderson RA; J Biol Chem 1996 20;271:32937-32943.

992. PolyA_pol: Poly A polymerase family
This family includes nucleic acid independent RNA polymerases, such as Poly(A) polymerase, which adds the poly (A) tail to mRNA EC:2.7.7.19. This family also includes the tRNA nucleotidyltransferase that adds the CCA to the 3' of the tRNA EC:2.7.7.25. Number of members: 31

[1] Medline: 93066242 Identification of the gene for an Escherichia coli poly(A) polymerase. Cao GJ, Sarkar N; Proc Natl Acad Sci U S A 1992;89:10380-10384.

993. Photosystem I psaA and psaB proteins signature (psaA_psaB)
PROSITE cross-reference(s)PS00419; PHOTOSYSTEM_I_PSAAB

Photosystem I (PSI) [1] is an integral membrane protein complex that uses light energy to mediate electron transfer from plastocyanin to ferredoxin. PSI is found in the chloroplast of plants and cyanobacteria. The electron transfer components of the reaction center of PSI are a primary electron donor P-700 (chlorophyll dimer) and five electron acceptors: A0 (chlorophyll), A1 (a phylloquinone) and three 4Fe-4S iron-sulfur centers: Fx, Fa, and Fb.

PsaA and psaB, two closely related proteins, are involved in the binding of P700, A0, A1, and Fx. psaA and psaB are both integral membrane proteins of 730 to 750 amino acids that seem to contain 11 transmembrane segments. The Fx 4Fe-4S iron-sulfur center is bound by four cysteines; two of these cysteines are provided by the psaA protein and the two others by psaB. The two cysteines in both proteins are proximal and located in a loop between the ninth and tenth transmembrane segments. A leucine zipper motif seems to be present [2] downstream of the cysteines and could contribute to dimerization of psaA/psaB.

The signature pattern for these proteins is based on the perfectly conserved region that includes the two iron-sulfur binding cysteines.
Consensus patternC-D-G-P-G-R-G-G-T-C [The two C's bind the iron-sulfur center]

[1]Golbeck J.H. Biochim. Biophys. Acta 895:167-204(1987).

[2]Webber A.N., Malkin R. FEBS Lett. 264:1-14(1990).

994. PSBH: Photosystem II 10 kDa phosphoprotein

This protein is phosphorylated in a light dependent reaction.

Number of members: 20

995. PsbJ

This family consists of the photosystem II reaction center protein PsbJ from plants and Cyanobacteria. In Synechocystis sp. PCC 6803 PsbJ regulates the number of photosystem II centers in thylakoid membranes, it is a predicted 4kDa protein with one membrane spanning domain [1]. Number of members: 20

[1] Medline: 93131892. Genetic and immunological analyses of the cyanobacterium Synechocystis sp. PCC 6803 show that the protein encoded by the psbJ gene regulates the number of photosystem II centers in thylakoid membranes. Lind LK, Shukla VK, Nyhus KJ, Pakrasi HB; J Biol Chem 1993;268:1575-1579.

996. PSBT: Photosystem II reaction centre T protein

The exact function of this protein is unknown. It probably consists of a single transmembrane spanning helix. The Swiss:P37256 protein, appears to be (i) a novel photosystem II subunit and (ii) required for maintaining optimal photosystem II activity under adverse growth conditions [1]. Number of members: 17

[1] Medline: 94298765. The chloroplast ycf8 open reading frame encodes a photosystem II polypeptide which maintains photosynthetic activity under adverse growth conditions. Monod C, Takahashi Y, Goldschmidt-Clermont M, Rochaix JD; EMBO J 1994;13:2747-2754.

997. PSI_8. PHOTOSYSTEM I REACTION CENTRE SUBUNIT VIII. Synonym(s)PSI-I. Gene name(s)PSAI. From Hordeum vulgare (Barley). Encoded on Chloroplast. Taxonomy

Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Hordeum.

MAY HELP IN THE ORGANIZATION OF THE PSAL SUBUNIT. BELONGS TO THE PSAI FAMILY.

[1] SEQUENCE FROM N.A. MEDLINE; 90036933. Scheller H.V., Okkels J.S., Hoej P.B., Svendsen I., Roepstorff P., Moeller B.L.; "The primary structure of a 4.0-kDa photosystem I polypeptide encoded by the chloroplast psaI gene."; J. Biol. Chem. 264:18402-18406(1989).

998. PSI_PsaJ: Photosystem I reaction centre subunit IX / PsaJ

This family consists of the photosystem I reaction centre subunit IX or PsaJ from various organisms including Synechocystis sp. (strain pcc 6803), Pinus thunbergii (green pine) and Zea mays (maize). PsaJ Swiss:P19443 is a small 4.4kDa, chloroplastal encoded, hydrophobic subunit of the photosystem I reaction complex its function is not yet fully understood [1]. PsaJ can be cross-linked to PsaF Swiss:P12356 and has a single predicted transmembrane domain it has a proposed role in maintaing PsaF in the correct orientation to allow for fast electron transfer from soluble donor proteins to P700+ [1]. Number of members: 18

[1] Medline: 99238330. A large fraction of PsaF is nonfunctional in photosystem I complexes lacking the PsaJ subunit. Fischer N, Boudreau E, Hippler M, Drepper F, Haehnel W, Rochaix JD; Biochemistry 1999;38:5546-5552.

[2] Medline: 93252282. Genes encoding eleven subunits of photosystem I from the thermophilic cyanobacterium Synechococcus sp. Muhlenhoff U, Haehnel W, Witt H, Herrmann RG; Gene 1993;127:71-78.

999. PSII. Protein namePHOTOSYSTEM II P680 CHLOROPHYLL A APOPROTEIN. Synonym(s)CP-47 PROTEIN. Gene name(s)PSBB. From Hordeum vulgare (Barley), Encoded on Chloroplast. Taxonomy Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Hordeum.

FUNCTION: THIS PROTEIN CONJUGATES WITH CHLOROPHYLL & CATALYZES THE PRIMARY LIGHT-INDUCED PHOTOCHEMICAL PROCESSES OF PHOTOSYSTEM II. SUBCELLULAR LOCATION: CHLOROPLAST THYLAKOID MEMBRANE. SIMILARITY: BELONGS TO THE PSBB / PSBC FAMILY.

09505352.061502

[1] SEQUENCE FROM N.A. STRAIN=CV. SABARLIS; MEDLINE; 89240047. Andreeva A.V., Buryakova A.A., Reverdatto S.V., Chakhmakhcheva O.G., Efimov V.A.; "Nucleotide sequence of the 5.2 kbp barley chloroplast DNA fragment, containing psbB-psbH-petB-petD gene cluster."; Nucleic Acids Res. 17:2859-2860(1989).

[2] SEQUENCE FROM N.A. STRAIN=CV. SABARLIS; MEDLINE; 92207253. Efimov V.A., Andreeva A.V., Reverdatto S.V., Chakhmakhcheva O.G.; "Photosystem II of rye. Nucleotide sequence of the psbB, psbC, psbE, psbF, psbH genes of rye and chloroplast DNA regions adjacent to them."; Bioorg. Khim. 17:1369-1385(1991).

[3] SEQUENCE OF 411-420. Hinz U.G.; "Isolation of the photosystem II reaction center complex from barley. Characterization by cicular dichroism spectroscopy and amino acid sequencing."; Carlsberg Res. Commun. 50:285-298(1985).

1000. QRPTase. Quinolinate phosphoribosyl transferase.

Quinolinate phosphoribosyl transferase (QPRTase) or nicotinate-nucleotide pyrophosphorylase EC:2.4.2.19 is involved in the de novo synthesis of NAD in both prokaryotes and eukaryotes. It catalyses the reaction of quinolinic acid with 5-phosphoribosyl-1-pyrophosphate (PRPP) in the presence of Mg2+ to give rise to nicotinic acid mononucleotide (NaMN), pyrophosphate and carbon dioxide [1,2]. Number of members: 26.

[1]Medline: 97169443. A new function for a common fold: the crystal structure of quinolinic acid phosphoribosyltransferase. Eads JC, Ozturk D, Wexler TB, Grubmeyer C, Sacchettini JC; Structure 1997;5:47-58.

[2]Medline: 96139309. The sequencing expression, purification, and steady-state kinetic analysis of quinolinate phosphoribosyl transferase from Escherichia coli. Bhatia R, Calvo KC; Arch Biochem Biophys 1996;325:270-278.

1001. R3H domain

The name of the R3H domain comes from the characteristic spacing of the most conserved arginine and histidine residues. The function of the domain is predicted to be binding ssDNA. Number of members: 28

[1]Medline: 99003905 The R3H motif: a domain that binds single-stranded nucleic acids. Grishin NV; Trends Biochem Sci 1998;23:329-330.

1002. recF protein signatures (RecF)

The prokaryotic protein recF [1,2] is a single-stranded DNA-binding protein which also probably binds ATP. RecF is involved in DNA metabolism; it is required for recombinational DNA repair and for induction of the SOS response. RecF is a protein of about 350 to 370 amino acid residues; there is a conserved ATP-binding site motif 'A' (P-loop) in the N-terminal section of the protein as well as two other conserved regions, one located in the central section, and the other in the C-terminal section. Signature patterns were derived from these two regions.

Consensus pattern [LIVM]-x(4)-[LIF]-x(6)-[LIF]-[LVF]-x-[GE]-[GSTAD]-[PA]- x(2)-R-R-x-[FYW]-[LIVMF]-D Sequences known to belong to this class detected by the pattern ALL.

Consensus pattern[LIVMFY](2)-x-D-x(2,3)-[SA]-[EH]-L-D-x(2)-[KRH]-x(3)-L Sequences known to belong to this class detected by the patternALL, except for T. palidum recF.

[1] Sandler S.J., Chackerian B., Li J.T., Clark A.J. Nucleic Acids Res. 20:839-845(1992).
[2] Alonso J.C., Fisher L.M.; Mol. Gen. Genet. 246:680-686(1995).

1003. RibD C-terminal domain (RibD_C)

The function of this domain is not known, but it is thought to be involved in riboflavin biosynthesis. This domain is found in the C terminus of RibD/RibG Swiss:P25539, in combination with dCMP_cyt_deam, as well as in isolation in some archaebacterial proteins Swiss:P95872.

Number of members: 21

1004. Ribosomal protein L16 signatures (Ribosomal_L16)

09565046.030601

Ribosomal protein L16 is one of the proteins from the large ribosomal subunit. In Escherichia coli, L16 is known to bind directly the 23S rRNA and to be located at the A site of the peptidyltransferase center. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups:

- Eubacterial L16.
- Algal and plant chloroplast L16.
- Cyanelle L16.
- Plant mitochondrial L16.

L16 is a protein of 133 to 185 amino-acid residues. As signature patterns, we selected two conserved regions in the central section of these proteins.

09555256.061501

Consensus pattern [KR](2)-x-[GSAC]-[KRQVA]-[LIVM]-W-[LIVM]-[KR]-[LIVM]-[LFY]-[AP] Sequences known to belong to this class detected by the pattern ALL.

Consensus patternR-M-G-x-[GR]-K-G-x(4)-[FWKR] Sequences known to belong to this class detected by the patternALL.

[1] Otaka E., Hashimoto T., Mizuta K., Suzuki K. Protein Seq. Data Anal. 5:301-313(1993).

1005. Ribosomal protein L32e signature (Ribosomal_L32E)

A number of eukaryotic and archaebacterial ribosomal proteins can be grouped on the basis of sequence similarities. One of these families consists of:

- Mammalian L32 [1].
- Drosophila RP49 [2].
- Trichoderma harzianum L32 [3].
- Yeast L32e (YBL092w).
- Archaebacterial L32e [4].

These proteins have 135 to 240 amino-acid residues. As a signature pattern, a stretch of about 20 residues located in the N-terminal part of these proteins was seleced.

Consensus patternF-x-R-x(4)-[KR]-x(2)-[KR]-[LIVMF]-x(3,5)-W-R-[KR]-x(2)-G Sequences known to belong to this class detected by the pattern ALL.

[1] Jacks C.M., Powaser C.B., Hackett P.B. Gene 74:565-570(1988).

[2] Aguade M. Mol. Biol. Evol. 5:433-441(1988).

[3] Lora J.M., Garcia I., Benitez T., Llobell A., Pintor-Toro J.A. Nucleic Acids Res. 21:3319-3319(1993).

[4] Arndt E., Scholzen T., Kroemer W., Hatakeyama T., Kimura M. Biochimie 73:657-668(1991).

1006. (Ribosomal_S3) Ribosomal protein S3 signature

PROSITE: PDOC00474. PROSITE cross-reference(s) PS00548; RIBOSOMAL_S3

Ribosomal protein S3 is one of the proteins from the small ribosomal subunit. In Escherichia coli, S3 is known to be involved in the binding of initiator Met-tRNA. It belongs to a family of ribosomal proteins which, on the basis of sequence similarities [1], groups:

-Eubacterial S3.
-Algal and plant chloroplast S3.
-Cyanelle S3.
-Archaebacterial S3.
-Plant mitochondrial S3.
-Vertebrate S3.
-Insect S3.
-Caenorhabditis elegans S3 (C23G10.3).
-Yeast S3 (Rp13).

S3 is a protein of 209 to 559 amino-acid residues. A conserved region located in the C-terminal section was selected as a signature pattern.

Consensus pattern[GSTA]-[KR]-x(6)-G-x-[LIVMT]-x(2)-[NQSCH]-x(1,3)-[LIVFCA]-x(3)-[LIV]-[DENQ]-x(7)-[LMT]-x(2)-G-x(2)-[GS]. Sequences known to belong to this class detected by the patternALL, except for some mitochondrial S3.

[1]Otaka E., Hashimoto T., Mizuta K. Protein Seq. Data Anal. 5:285-300(1993).

1007. RimM - RimM

09515325.061500

The RimM protein is essential for efficient processing of 16S rRNA [1]. The RimM protein was shown to have affinity for free ribosomal 30S subunits but not for 30S subunits in the 70S ribosomes [1]. Number of members: 14.

[1]Medline: 98083058. RimM and RbfA are essential for efficient processing of 16S rRNA in Escherichia coli. Bylund GO, Wipemo LC, Lundberg LA, Wikstrom PM; J Bacteriol 1998;180:73-82.

1008. RNA_pol_A - RNA polymerase alpha subunit

-!- RNA polymerases catalyse the DNA dependent polymerisation of RNA. Prokaryotes contain a single RNA polymerase compared to three in eukaryotes (not including mitochondrial and chloroplast polymerases).

-!- Members of this family include: A subunit from eukaryotes, gamma subunit from cyanobacteria, beta' subunit from eubacteria, A' subunit from archaebacteria, B" from chloroplasts. Number of members: 139.

09595326.061600

[1]Medline: 97066998. Structural modules of the large subunits of RNA polymerase. Introducing archaebacterial and chloroplast split sites in the beta and beta' subunits of Escherichia coli RNA polymerase. Severinov K, Mustaev A, Kukarin A, Muzzin O, Bass I, Darst SA, Goldfarb A; J Biol Chem 1996;271:27969-27974.

1009. RuBisCO_large - Ribulose bisphosphate carboxylase large chain active site

PROSITE: PDOC00142; PROSITE cross-reference(s) PS00157; RUBISCO_LARGE

Ribulose bisphosphate carboxylase (EC 4.1.1.39) (RuBisCO) [1,2] catalyzes the initial step in Calvin's reductive pentose phosphate cycle in plants as well as purple and green bacteria. It consists of a large catalytic unit and a small subunit of undetermined function. In plants, the large subunit is coded by the chloroplastic genome while the small subunit is encoded in the nuclear genome. Molecular activation of RuBisCO by CO2 involves the formation of a carbamate with the epsilon-amino group of a conserved lysine residue. This carbamate is stabilized by a magnesium ion. One of the ligands of the magnesium ion is an aspartic acid residue close to the active site lysine [3]. A pattern was developed which includes both the active site residue and the metal ligand, and which is specific to RuBisCO large chains.

Consensus patternG-x-[DN]-F-x-K-x-D-E [K is the active site residue] [The second D is a magnesium ligand]. Sequences known to belong to this class detected by the patternALL, except for Cheilopleuria biscuspis RuBisCO.

[1]Miziorko H.M., Lorimer G.H. Annu. Rev. Biochem. 52:507-535(1983).
[2]Akazawa T., Takabe T., Kobayashi H. Trends Biochem. Sci. 9:380-383(1984).
[3]Andersson I., Knight S., Schneider G., Lindqvist Y., Lundqvist T., Branden C.-I., Lorimer G.H. Nature 337:229-234(1989).

09565326.071801

1010. Rve - Integrase core domain

Integrase mediates integration of a DNA copy of the viral genome into the host chromosome. Integrase is composed of three domains. The amino-terminal domain is a zinc binding domain Integrase_Zn. This domain is the central catalytic domain. The carboxyl terminal domain that is a non-specific DNA binding domain integrase. The catalytic domain acts as an endonuclease when two nucleotides are removed from the 3' ends of the blunt-ended viral DNA made by reverse transcription. This domain also catalyses the DNA strand transfer reaction of the 3' ends of the viral DNA to the 5' ends of the integration site [1]. Number of members: 694.

[1]Medline: 95099322. Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. Dyda F, Hickman AB, Jenkins TM, Engelman A, Craigie R, Davies DR; Science 1994;266:1981-1986.

1011. (SBP_bac_3) Bacterial extracellular solute-binding proteins, family 3 signature PROSITE: PDOC00798. PROSITE cross-reference(s) PS01039; SBP_BACTERIAL_3

Bacterial high affinity transport systems are involved in active transport of solutes across the cytoplasmic membrane. The protein components of these traffic systems include one or two transmembrane protein components, one or two membrane-associated ATP-binding proteins (ABC transporters; see <PDOC00185>) and a high affinity periplasmic solute-binding protein. The later are thought to bind the substrate in the vicinity of the inner membrane, and to transfer it to a complex of inner membrane proteins for concentration into the cytoplasm.

In gram-positive bacteria which are surrounded by a single membrane and have therefore no periplasmic region the equivalent proteins are bound to the membrane via an N-terminal lipid anchor. These homolog proteins do not play an integral role in the transport process per se, but probably serve as receptors to trigger or initiate translocation of the solute throught the membrane by binding to external sites of the integral membrane proteins of the efflux system.

In addition at least some solute-binding proteins function in the initiation of sensory transduction pathways.

On the basis of sequence similarities, the vast majority of these solute-binding proteins can be grouped [1] into eight families of clusters, which generally correlate with the nature of the solute bound.

Family 3 groups together specific amino acids and opine-binding periplasmic proteins and a periplasmic homolog with catalytic activity:

-Histidine-binding protein (gene hisJ) of Escherichia coli and related bacteria. An homologous lipoprotein exists in Neisseria gonorrhoeae.

-Lysine/arginine/ornithine-binding proteins (LAO) (gene argT) of Escherichia coli and related bacteria are involved in the same transport system than hisJ. Both solute-binding proteins interact with a common membrane-bound receptor hisP of the binding protein dependent transport system HisQMP.

-Glutamine-binding proteins (gene glnH) of Escherichia coli and Bacillus stearothermophilus.

-Glutamate-binding protein (gene gluB) of Corynebacterium glutamicum.

-Arginine-binding proteins artI and artJ of Escherichia coli.

-Nopaline-binding protein (gene nocT) from Agrobacterium tumefaciens.

-Octopine-binding protein (gene occT) from Agrobacterium tumefaciens.

-Major cell-binding factor (CBF1) (gene: peb1A) from Campylobacter jejuni.

-Bacteroides nodosus protein aabA.

-Cyclohexadienyl/arogenate dehydratase of Pseudomonas aeruginosa, a periplasmic enzyme which forms an alternative pathway for phenylalanine biosynthesis.

-Escherichia coli protein fliY.

-Vibrio harveyi protein patH.

-Escherichia coli hypothetical protein ydhW.

-Bacillus subtilis hypothetical protein yckB.

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-Bacillus subtilis hypothetical protein yckK.

The signature pattern is located near the N-terminus of the mature proteins.
Consensus patternG-[FYIL]-[DE]-[LIVMT]-[DE]-[LIVMF]-x(3)-[LIVMA]-[VAGC]-x(2)-[LIVMAGN]
Sequences known to belong to this class detected by the patternALL.

[1]Tam R., Saier M.H. Jr. Microbiol. Rev. 57:320-346(1993).

1012. Sec7 - Sec7 domain
The Sec7 domain is a guanine-nucleotide-exchange-factor (GEF)for the arf family [2].
Number of members: 32.

09555526.061901

[1]Medline: 98169075. Structure of the Sec7 domain of the Arf exchange factor. ARNO. Cherfils J, Menetrey J, Mathieu M, Le Bras G, Robineau S, Beraud-Dufour S, Antonny B, Chardin P; Nature 1998;392:101-105.
[2]Medline: 97100951. A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. Chardin P, Paris S, Antonny B, Robineau S, Beraud-Dufour S, Jackson CL, Chabre M. Nature 1996;384:481-484.

1013. SecA_protein. SecA protein, amino terminal region
SecA protein binds to the plasma membrane where it interacts with proOmpA to support translocation of proOmpA through the membrane. SecA protein achieves this translocation, in association with SecY protein, in an ATP dependent manner. SecA possesses the ATPase activity. The carboxyl terminus has similarity with the helicase carboxyl terminus. See Ribosomal_L5. Number of members: 45.

[1]Medline: 98309858. Amino-terminal region of SecA is involved in the function of SecG for protein translocation into Escherichia coli membrane vesicles. Mori H, Sugiyama H, Yamanaka M, Sato K, Tagaya M, Mizushima S; J Biochem (Tokyo) 1998;124:122-129.
[2]Medline: 89251629. SecA protein hydrolyzes ATP and is an essential component of the protein translocation ATPase of Escherichia coli. Lill R, Cunningham K, Brundage LA, Ito K, Oliver D, Wickner W; EMBO J 1989;8:961-966.

1014. Seedstore_2S - 2S seed storage family

Members of this family are composed of two chains (both included in the alignment), these are co-translated and later cleaved. The two chains are disulphide linked together. Number of members: 27.

[1]Medline: 97121264. 1H NMR assignment and global fold of napin BnIb, a representative 2S albumin seed protein. Rico M, Bruix M, Gonzalez C, Monsalve RI, Rodriguez R; Biochemistry 1996;35:15672-15682.

1015. Smr - Smr domain

This family includes the Smr (Small MutS Related) proteins, and the C-terminal region of the MutS2 protein. It has been suggested that this domain interacts with the MutS1 Swiss:P23909 protein in the case of Smr proteins and with the N-terminal MutS related region of MutS2 Swiss:P94545 [1]. Number of members: 14.

09555326.052601

[1]Medline: 10431172. Smr: a bacterial and eukaryotic homologue of the C-terminal region of the MutS2 family. Moreira D, Philippe H; Trends Biochem Sci 1999;24:298-300.

1016. (SSF) Sodium:solute symporter family signatures and profile

PROSITE: PDOC00429. PROSITE cross-reference(s)PS00456; NA_SOLUT_SYMP_1 PS00457; NA_SOLUT_SYMP_2 PS50283; NA_SOLUTE_SYMP_3

It has been shown [1,2] that integral membrane proteins that mediate the intake of a wide variety of molecules with the concomitant uptake of sodium ions (sodium symporters) can be grouped, on the basis of sequence and functional similarities into a number of distinct families. One of these families is known as the sodium:solute symporter family (SSF) and currently consists of the following proteins:

-Mammalian Na+/glucose co-transporter.

-Mammalian Na+/myo-inositol co-transporter.

-Mammalian Na+/nucleoside co-transporter.

-Mammalian Na+/neutral amino acid co-transporter.

-Escherichia coli Na+/proline symporter (gene putP).

-Escherichia coli Na+/pantothenate symporter (gene panF).

-Escherichia coli hypothetical protein yidK.

-Escherichia coli hypothetical protein yjcG.

-Bacillus subtilis hypothetical protein ywcA (ipa-31R).

These integral membrane proteins are predicted to comprise at least ten membrane spanning domains. Two conserved regions were selected as signature patterns; the first one is located in the fourth transmembrane region and the second one in a loop between two transmembrane regions in the C-terminal part of these proteins.

Consensus pattern[GS]-x(2)-[LIY]-x(3)-[LIVMFYWSTAG](10)-[LIY]-[TAV]-x(2)-G-G-[LMF]-x-[SAP]. Sequences known to belong to this class detected by the patternALL.
Consensus pattern[GAST]-[LIVM]-x(3)-[KR]-x(4)-G-A-x(2)-[GAS]-[LIVMGS]-[LIVMW]-[LIVMGAT]-G-x-[LIVMGA] Sequences known to belong to this class detected by the patternALL, except for E.coli yidK.
Note this documentation entry is linked to both a signature pattern and a profile. As the profile is much more sensitive than the pattern, you should use it if you have access to the necessary software tools to do so.

[1]Reizer J., Reizer A., Saier M.H. Jr. Res. Microbiol. 141:1069-1072(1991).
[2]Reizer J., Reizer A., Saier M.H. Jr. Biochim. Biophys. Acta 1197:133-136(1994).

09595365.061502

1017. SurE - Survival protein SurE
E. coli cells with the surE gene disrupted are found to survive poorly in stationary phase [1]. It is suggested that SurE may be involved in stress response. Yeast also contains a member of the family Swiss:P38254. Swiss:P30887 can complement a mutation in acid phosphatase, suggesting that members of this family could be phosphatases. Number of members: 17.

[1]Medline: 95014035. A new gene involved in stationary-phase survival located at 59 minutes on the Escherichia coli chromosome. Li C, Ichikawa JK, Ravetto JJ, Kuo HC, Fu JC, Clarke S; J Bacteriol 1994;176:6015-6022.
[2]Medline: 93046805. Complementation of Saccharomyces cerevisiae acid phosphatase mutation by a genomic sequence from the yeast Yarrowia lipolytica identifies a new phosphatase. Treton BY, Le Dall MT, Gaillardin CM; Curr Genet 1992;22:345-355.

1018. Synuclein - Synuclein

There are three types of synucleins in humans, these are called alpha, beta and gamma. Alpha synuclein has been found mutated in families with autosomal dominant Parkinson's disease. A peptide of alpha synuclein has also been found in amyloid plaques in Alzheimer's patients. Number of members: 12.

[1]Medline: 98424410. The synuclein family. Lavedan C; Genome Res 1998;8:871-880.

1019. (T-box) T-box domain signatures

PROSITE: PDOC00972. PROSITE cross-reference(s) PS01283; TBOX_1 PS01264; TBOX_2

A number of eukaryotic DNA-binding proteins contain a domain of about 170 to 190 amino acids known as the T-box domain [1,2,3] and which probably binds DNA. The T-box has first been found in the mice T locus (Brachyury) protein, a transcription factor involved in mesoderm differentiation. It has since been found in the following proteins:

-Vertebrate and invertebrate homologs of the T protein.
-Mammalian proteins TBX1 to TBX6.
-Mammalian protein TBR1 which is expressed specifically in brain.
-Xenopus laevis eomesodermin (eomes).
-Xenopus laevis Vegt (or Antipodean), a transcription factor that activates the expression of wnt-8, eomes and Brachyury.
-Chicken TbxT.
-Drosophila protein optomotor-blind (omb).
-Drosophila protein brachyenteron (byn) (also known as Trg), which is required for the specification of the hindgut and anal pads.
-Drosophila protein H15.
-Caenorhabditis elegans protein tbx-12.
-Caenorhabditis elegans hypothetical proteins F21H11.3, F40H6.4, T07C4.2, T07C4.6 and ZK177.10.

Two conserved regions were selected as signature patterns for the T-domain. The first region corresponds to the N-terminal of the domain and the second one to the central part.

Consensus patternL-W-x(2)-[FC]-x(3,4)-[NT]-E-M-[LIV](2)-T-x(2)-G-[RG]-[KRQ]

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Sequences known to belong to this class detected by the patternALL, except for C.elegans ZK177.10.

Consensus pattern[LIVMYW]-H-[PADH]-[DEN]-[GS]-x(3)-G-x(2)-W-M-x(3)-[IVA]-x- F

Sequences known to belong to this class detected by the patternALL, except for C.elegans tbx-12, ZK177.10 and Drosophila H15.

[1]Bollag R.J., Siegfried Z., Cebra-Thomas J.A., Garvey N., Davison E.M., Silver L.M. Nat. Genet. 7:383-389(1994).

[2] Agulnik S.I., Garvey N., Hancock S., Ruvinsky I., Chapman D.L., Agulnik I., Bollag R.J., Papaioannou V.E., Silver L.M. Genetics 144:249-254(1996).

[3]Papaioannou V.E. Trends Genet. 13:212-213(1997).

1020. Toprim - Toprim domain

This is a conserved region from DNA primase. This corresponds to the Toprim domain common to DnaG primases, topoisomerases, OLD family nucleases and RecR proteins [1]. Both DnaG motifs IV and V are present in the alignment, the DxD (V) motif may be involved in Mg2+ binding and mutations to the conserved glutamate (IV) completely abolish DnaG type primase activity [1]. DNA primase EC:2.7.7.6 is a nucleotidyltransferase it synthesizes the oligoribonucleotide primers required for DNA replication on the lagging strand of the replication fork; it can also prime the leading stand and has been implicated in cell division [2]. Number of members: 133.

[1]Medline: 98391745. Toprim--a conserved catalytic domain in type IA and II topoisomerases, DnaG-type primases, OLD family nucleases and RecR proteins. Aravind L, Leipe DD, Koonin EV; Nucleic Acids Res 1998;26:4205-4213.

[2]Medline: 97368180. Cloning and analysis of the dnaG gene encoding Pseudomonas putida DNA primase. Szafranski P, Smith CL, Cantor CR; Biochim Biophys Acta 1997;1352:243-248.

[3]Medline: 94124015. The Haemophilus influenzae dnaG sequence and conserved bacterial primase motifs. Versalovic J, Lupski JR; Gene 1993;136:281-286.

1021. TraB - TraB family

pAD1 is a hemolysin/bacteriocin plasmid originally identified in Enterococcus faecalis DS16. It encodes a mating response to a peptide sex pheromone, cAD1, secreted by recipient bacteria. Once the plasmid pAD1 is acquired, production of the pheromone ceases--a trait related in part to a determinant designated traB. However a related protein is found in C. elegans Swiss:Q94217, suggesting that members of the TraB family have some more general function. Number of members: 12.

[1]Medline: 94302142. Characterization of the determinant (traB) encoding sex pheromone shutdown by the hemolysin/bacteriocin plasmid pAD1 in Enterococcus faecalis. An FY, Clewell DB; Plasmid 1994;31:215-221.

09553609.051502

1022. (Transpo_mutator) Transposases, Mutator family, signature
PROSITE: PDOC00770. PROSITE cross-reference(s) PS01007;
TRANSPOSASE_MUTATOR

Autonomous mobile genetic elements such as transposon or insertion sequences (IS) encode an enzyme, called transposase, required for excising and inserting the mobile element. On the basis of sequence similarities, transposases can be grouped into various families. One of these families has been shown [1,2,3,E1] to consist of transposases from the following elements:

-Mutator from Maize.
-Is1201 from Lactobacillus helveticus.
-Is905 from Lactococcus lactis.
-Is1081 from Mycobacterium bovis.
-Is6120 from Mycobacterium smegmatis.
-Is406 from Pseudomonas cepacia.
-IsRm3 from Rhizobium meliloti.
-IsRm5 from Rhizobium meliloti.
-Is256 from Staphylococcus aureus.
-IsT2 from Thiobacillus ferrooxidans.

The maize Mutator transposase (MudrA) is a protein of 823 amino acids; the bacterial transposases listed above are proteins of 300 to 420 amino acids. These proteins contain a conserved domain of about 130 residues; a signature pattern was derived from the most conserved part of this domain.

Consensus patternD-x(3)-G-[LIVMF]-x(6)-[STAV]-[LIVMFYW]-[PT]-x-[STAV]-x(2)-[QR]-x-C-x(2)-H. Sequences known to belong to this class detected by the patternALL.

[1]Eisen J.A., Benito M.-I., Walbot V. Nucleic Acids Res. 22:2634-2636(1994).
[2]Guilhot C., Gicquel B., Davies J., Martin C. Mol. Microbiol. 6:107-113(1992).
[3]Wood M.S., Byrne A., Lessie T.G. Gene 105:101-105(1991).

1023. Transposase_8 - Transposase

Transposase proteins are necessary for efficient DNA transposition. This family consists of various E. coli insertion elements and other bacterial transposases some of which are members of the IS3 family. Number of members: 58.

[1]Medline: 97324595. Genetic organization and transposition properties of IS511. D. A. Mullin, D. L. Zies, A. H. Mullin, N. Caballera & B. Ely; Mol Gen Genet 1997;254:456-463.
[2]Medline: 97128810. The use of an improved transposon mutagenesis system for DNA sequencing leads to the characterization of a new insertion sequence of Streptomyces lividans 66. J. Fischer, H. Maier, P. Viell & J. Altenbuchner; Gene 1996;180:81-89.
[3]Medline: 97074647. Identification and nucleotide sequence of Rhizobium meliloti insertion sequence ISRm6, a small transposable element that belongs to the IS3 family. S. Zekri & N. Toro; Gene 1996;175:43-48.

1024. tRNA_int_endo - tRNA intron endonuclease

Members of this family cleave pre tRNA at the 5' and 3' splice sites to release the intron EC:3.1.27.9. Number of members: 8.

[1]Medline: 97344075. Properties of H. volcanii tRNA intron endonuclease reveal a relationship between the archaeal and eucaryal tRNA intron processing systems. Kleman-Leyer K, Armbruster DW, Daniels CJ; Cell 1997;89:839-847.

1025. Urease - Urease signatures

PROSITE: PDOC00133PROSITE cross-reference(s) PS01120; UREASE_1 PS00145; UREASE_2

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Urease (EC 3.5.1.5) is a nickel-binding enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia [1]. Historically, it was the first enzyme to be crystallized (in 1926). It is mainly found in plant seeds, microorganisms and invertebrates. In plants, urease is a hexamer of identical chains. In bacteria [2], it consists of either two or three different subunits (alpha, beta and gamma).

Urease binds two nickel ions per subunit; four histidine, an aspartate and a carbamated-lysine serve as ligands to these metals; an additional histidine is involved in the catalytic mechanism [3].

As signatures for this enzyme, a region that contains two histidine that bind one of the nickel ions and the region of the active site histidine was selected.

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Consensus pattern T-[AY]-[GA]-[GAT]-[LIVM]-D-x-H-[LIVM]-H-x(3)-P [The two H's bind nickel].Sequences known to belong to this class detected by the patternALL.
Consensus pattern[LIVM](2)-[CT]-H-[HN]-L-x(3)-[LIVM]-x(2)-D-[LIVM]-x-F-A [H is the active site residue]. Sequences known to belong to this class detected by the patternALL.

[1]Takishima K., Suga T., Mamiya G. Eur. J. Biochem. 175:151-165(1988).
[2]Mobley H.L.T., Husinger R.P. Microbiol. Rev. 53:85-108(1989).
[3]Jabri E., Carr M.B., Hausinger R.P., Karplus P.A. Science 268:998-1004(1995).

1026. Urease_beta - Urease beta subunit.
This subunit is known as alpha in Heliobacter. Number of members: 35.

[1]Medline: 95273988. The crystal structure of urease from Klebsiella aerogenes. Jabri E, Carr MB, Hausinger RP, Karplus PA; Science 1995;268:998-1004.

1027. UvrD-helicase - UvrD/REP helicase
The Rep family helicases are composed of four structural domains. The Rep family function as dimers. REP helicases catalyse ATP dependent unwinding of double stranded DNA to single stranded DNA. Swiss:P23478, Swiss:P08394 have large insertions near to the carboxy-terminus relative to other members of the family. Number of members: 52.

[1] Medline: 97433075. Major domain swiveling revealed by the crystal structures of complexes of E. coli Rep helicase bound to single-stranded DNA and ADP. Korolev S, Hsieh J, Gauss GH, Lohman TM, Waksman G; Cell 1997;90:635-647.

1028. V-type ATPase 116kDa subunit family (V_ATPase_sub_a)

This family consists of the 116kDa V-type ATPase (vacuolar (H+)-ATPases) subunits, as well as V-type ATP synthase subunit i. The V-type ATPases family are proton pumps that acidify intracellular compartments in eukaryotic cells for example yeast central vacuoles, clathrin-coated and synaptic vesicles. They have important roles in membrane trafficking processes [1]. The 116kDa subunit (subunit a) in the V-type ATPase is part of the V0 functional domain responsible for proton transport. The a subunit is a transmembrane glycoprotein with multiple putative transmembrane helices t has a hydrophilic amino terminal and a hydrophobic carboxy terminal [1,2]. It has roles in proton transport and assembly of the V-type ATPase complex [1,2]. This subunit is encoded by two homologous gene in yeast VPH1 and STV1 [2].
Number of members: 27

[1] Forgac M; Medline: 99240666 “Structure and properties of the vacuolar (H+)-ATPases.” J Biol Chem 1999;274:12951-12954.
[2] Forgac M; Medline: 99270697 “Structure and properties of the clathrin-coated vesicle and yeast vacuolar V-ATPases.” J Bioenerg Biomembr 1999;31:57-65.

1029. Viral (Superfamily 1) RNA helicase (Viral_helicase1)
Number of members: 260

[1] Koonin EV, Dolja VV; Medline: 94094568 “Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences.” Crit Rev Biochem Mol Biol 1993;28:375-430.

1030. Vesicular monoamine transporter (VMAT)

09595395.051502

This family consists of various vesicular amine transporters with 12 transmembrane helices. These included vesicular acetylcholine transporters (VAChT) [3], and vesicular monoamine transporters (VMATs) [1,2] isoforms 1 adrenal and 2 brain (VMAT1 and VMAT2).

These proteins transport biogenic amines into synaptic vesicles or chromaffin granules [4]. VMATs pack monoamine neurotransmitters into secretary vesicles for regulated exocytotic release, they also protect against the parkinsonian neurotoxins MPP+ by transporting it into vesicles preventing it from acting on mitochondria [1].

Also in the family is C. elegans UNC-17 a putative vesicular acetylcholine transporter mutations in UNC-17 cause impaired neuromuscular function, giving rise to jerky or uncoordinated movement, [4].

Number of members: 15

[1] Krantz DE, Peter D, Liu Y, Edwards RH; Medline: 97197857 “Phosphorylation of a vesicular monoamine transporter by casein kinase II.” J Biol Chem 1997;272:6752-6759.

[2] Erickson JD, Varoqui H, Schafer MK, Modi W, Diebler MF, Weihe E, Rand J, Eiden LE, Bonner TI, Usdin TB; Medline: 94350930 “Functional identification of a vesicular acetylcholine transporter and its expression from a ‘cholinergic’ gene locus.” J Biol Chem 1994;269:21929-21932.

[3] Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E; Medline: 96209876 “Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter.” Proc Natl Acad Sci U S A 1996;93:5166-5171.

[4] Alfonso A, Grundahl K, Duerr JS, Han HP, Rand JB; Medline: 3342494 “The Caenorhabditis elegans unc-17 gene: a putative vesicular acetylcholine transporter.” Science 1993;261:617-619.

1031. WW/rsp5/WWP domain signature and profile. Cross-reference(s): PS01159; WW_DOMAIN_1; PS50020; WW_DOMAIN_2

The WW domain [1-4,E1] (also known as rsp5 or WWP) has been originally discovered as a short conserved region in a number of unrelated proteins, among them dystrophin, the gene responsible for Duchenne muscular dystrophy. The domain, which spans about 35 residues, is repeated up to 4 times in some proteins. It has been shown [5] to bind proteins with particular proline-motifs, [AP]-P-P-[AP]-Y, and thus resembles somewhat SH3 domains. It appears to contain beta-strands grouped around four conserved aromatic positions; generally Trp. The name WW or WWP derives from the presence of these Trp as well as that of a conserved Pro. It is frequently associated with other domains typical for proteins in signal transduction processes.

Proteins containing the WW domain are listed below.

--Dystrophin, a multidomain cytoskeletal protein. Its longest alternatively spliced form consists of an N-terminal actin-binding domain, followed by 24 spectrin-like repeats, a cysteine-rich calcium-binding domain and a C-terminal globular domain. Dystrophin form tetramers and is thought to have multiple functions including involvement in membrane stability, transduction of contractile forces to the extracellular environment and organization of membrane specialization. Mutations in the dystrophin gene lead to muscular dystrophy of Duchenne or Becker type. Dystrophin contains one WW domain C-terminal of the spectrin-repeats.

--Utrophin, a dystrophin-like protein of unknown function.

--Vertebrate YAP protein is a substrate of an unknown serine kinase. It binds to the SH3 domain of the Yes oncoprotein via a proline-rich region. This protein appears in alternatively spliced isoforms, containing either one or two WW domains [6].

--Mouse NEDD-4 plays a role in the embryonic development and differentiation of the central nervous system. It contains 3 WW modules followed by a HECT domain. The human ortholog contains 4 WW domains, but the third WW domain is probably spliced resulting in an alternate NEDD-4 protein with only 3 WW modules [3].

--Yeast RSP5 is similar to NEDD-4 in its molecular organization. It contains an N-terminal C2 domain (see <PDOC00380>), followed by a histidine-rich region, 3 WW domains and a HECT domain.

--Rat FE65, a transcription-factor activator expressed preferentially in liver. The activator domain is located within the N-terminal 232 residues of FE65, which also contain the WW domain.

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--Yeast ESS1/PTF1, a putative peptidyl prolyl cis-trans isomerase from family ppiC (see <PDOC00840>). A related protein, dodo (gene dod) exists in Drosophila and in mammals (gene PIN1).

--Tobacco DB10 protein. The WW domain is located N-terminal to the region with similarity to ATP-dependent RNA helicases.

--IQGAP, a human GTPase activating protein acting on ras. It contains an N-terminal domain similar to fly muscle mp20 protein and a C-terminal ras GTPase activator domain.

--Yeast pre-mRNA processing protein PRP40, Caenorhabditis elegans ZK1098.1 and fission yeast SpAC13C5.02 are related proteins with similarity to MYO2-type myosin, each containing two WW-domains at the N-terminus.

--Caenorhabditis elegans hypothetical protein C38D4.5, which contains one WW module, a PH domain (see <PDOC50003>) and a C-terminal phosphatidylinositol 3-kinase domain.

--Yeast hypothetical protein YFL010c.

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For the sensitive detection of WW domains, a profile was developed which spans the whole homology region as well as a pattern.

Description of pattern(s) and/or profile(s):

Consensus patternW-x(9,11)-[VFY]-[FYW]-x(6,7)-[GSTNE]-[GSTQCR]-[FYW]-x(2)-P.

[1] Bork P., Sudol M. Trends Biochem. Sci. 19:531-533(1994).

[2] Andre B., Springael J.Y. Biochem. Biophys. Res. Commun. 205:1201-1205(1994).

[3] Hofmann K.O., Bucher P. FEBS Lett. 358:153-157(1995).

[4] Sudol M., Chen H.I., Bougeret C., Einbond A., Bork P. FEBS Lett. 369:67-71(1995).

[5] Chen H.I., Sudol M. Proc. Natl. Acad. Sci. U.S.A. 92:7819-7823(1995).

[6] Sudol M., Bork P., Einbond A., Kastury K., Druck T., Negrini M., Huebner K., Lehman D. J. Biol. Chem. 270:14733-14741(1995).

1032. XPA protein signatures. cross-reference(s): XPA_1 PROSITE PS00752; PS00753;XPA_2.

Xeroderma pigmentosum (XP) [1] is a human autosomal recessive disease, characterized by a high incidence of sunlight-induced skin cancer. People's skin cells with this condition are hypersensitive to ultraviolet light, due to defects in the incision step of DNA excision repair. There are a minimum of seven genetic complementation groups involved in this pathway: XP-A to XP-G. XP-A is the most severe form of the disease and is due to defects in a 30 Kd nuclear protein called XPA (or XPAC) [2].

The sequence of the XPA protein is conserved from higher eukaryotes [3] to yeast (gene RAD14) [4]. XPA is a hydrophilic protein of 247 to 296 amino-acid residues which has a C4-type zinc finger motif in its central section.

Two signature were developed patterns for XPA proteins. The first corresponds to the zinc finger region, the second to a highly conserved region located some 12 residues after the zinc finger region.

Consensus patternC-x-[DE]-C-x(3)-[LIVMF]-x(1,2)-D-x(2)-L-x(3)-F-x(4)-C-x(2)-C
Consensus pattern[LIVM](2)-T-[KR]-T-E-x-K-x-[DE]-Y-[LIVMF](2)-x-D-x-[DE]

[1] Tanaka K., Wood R.D. Trends Biochem. Sci. 19:83-86(1994).
[2] Miura N., Miyamoto I., Asahina H., Satokata I., Tanaka K., Okada Y. J. Biol. Chem. 266:19786-19789(1991).
[3] Shimamoto T., Kohno K., Tanaka K., Okada Y. Biochem. Biophys. Res. Commun. 181:1231-1237(1991).
[4] Bankmann M., Prakash L., Prakash S. Nature 355:555-558(1992).

1033. YCF9

This family consists of the hypothetical protein product of the YCF9 gene from chloroplasts and cyanobacteria. Number of members: 16

1034. (DUF15)

It is highly conserved between eubacteria and eukaryotes.

Number of members: 30

1035. Lumenal portion of Cytochrome b559, alpha (gene psbE) subunit. (cytochr_b559a)

This family is the lumenal portion of cytochrome b559 alpha chain, matches to this family should be accompanied by a match to the cytochr_b559 family also. The Prosite pattern pattern matches the transmembrane region of the cytochrome b559 alpha and beta subunits.
Number of members: 16

A. Asparaginase 2

Asparaginase II (L-asparagine aminohydrolase II) is an extracellular protein that may be associated with the cell wall and whose expression is affected by the availability of nitrogen. Asparaginase II catalyzes the reaction of L-Asparagine + H_2O = L-Aspartate + NH_3. As many leukemias have high requirements for aspartic acid, asparaginase II proteins are useful as reagents for screening compounds for activity as leukemia chemotherapy products. Asparaginase II protein can also be over- or under-expressed to alter amino acid content in plant tissues or to modify nitrogen fixation and/or nitrogen metabolism in plants.

Ref: Bon et al. (1997) Appl Biochem Biotechnol 63-65: 203-12

B. Chloroa b-bind

Chlorophyll a-b binding proteins are located in the thylakoid membranes of the chloroplast and bind chlorophyll a and chlorophyll b, thereby triggering a chemical reaction (photosynthesis). These proteins are useful in controlling the rate, efficiency and/or output of photosynthesis. Overexpression of chlorophyll a-b binding proteins is expected to increase the rate of photosynthesis.

Ref: Leutwiler et al. (1986) Nucleic Acids Res 14: 4051-64
Brandt et al. (1992) Plant Mol Biol 19: 699-703

C. DMRL synthase

DMRL Synthase (6,7-Dimethyl-8-Ribityllumazine Synthase) catalyzes the last step in riboflavin (Vitamin B_2) synthesis, condensing 5-amino-6-(1'-D)-ribityl-amino-2,4(1H, 3H)-Pyrimidinedione with L-3,4-Dihydroxy-2-Butanone 4-Phosphate producing 6,7-Dimethyl-8-(1-D-Ribityl)Luminazine . The enzyme forms a homopentamer. Engineering of these proteins or those with homologous sequences/structures may allow control of the amounts of vitamin B_2 available in plants and/or accumulation of pigment, as well as altering reactions requiring hydrogen ion carriers/transmitters.

Ref: Garcia-Ramirez et al. (1995) J Biol Chem **270**: 23801-7

D. E1_N

These proteins are ATP-dependent DNA helicases that are required for initiation of viral DNA replication. They form a complex with the viral E2 protein. The E1-E2 complex binds to the replication origin that contains binding sites for both proteins. The majority of sequences known for this group of proteins are from various papillomaviruses, a type of double stranded DNA virus. In plants, the prototype double stranded DNA virus is Cauliflower Mosaic virus (CaMV). Manipulation of these proteins, especially to produce variant proteins that form non-productive complexes, enables production of plants that are resistant to infection by double stranded DNA viruses.

Ref: Yang et al. (1993) PNAS USA **90**: 5086-90
Ustav and Stenlund (1991) EMBO J **10**: 449-57
Callaway et al. (1996) Mol Plant Microbe Interact 9: 810-8

E. EF1_G

Elongation Factor-1 is composed of four subunits: alpha, beta, delta and gamma. Gamma subunits are presumed to play a role in anchoring the complex to other cellular components. Studies of EF-1 genes in plants suggests that different forms of the EF-1 subunits may be expressed in particular organs or in response to stress. Manipulation of the activity of these proteins, either by altered expression level or by structural mutation, may result in the accumulation of a particular protein in a chosen organ or allow production of particular proteins during stress conditions.

Ref: Kinzy et al. (1994) NAR 22: 2703-7
Dunn et al. (1993) Plant Mol Biol 23: 221-5
Aguilar et al. (1991) Plant Mol Biol 17: 351-60

F. ENV_polyprotein

This family comprises the envelope or coat proteins known from a number of different retroviruses. In mammalian species, retroviruses are responsible for diseases such as leukemia and HIV. In plants, retroviruses are known in both monocot (e.g. Zeon-1) and dicot (e.g. Arabidopsis and tobacco) species and have been shown to induce mutant alleles at new loci. Engineering of plant ENV proteins may allow mobilization or targeting of endogenous or introduced retroviruses, in essence generating a new method for mutant production, gene tagging and the like.

Ref: Mamoun et al (1990) J Virol 64: 4180-8
Grandbastien et al. (1989) Nature 337: 376-80
Wright and Voytas (1998) Genetics 149: 703-15

G. Glycosyl_hydr9

Proteins having this domain (previously known as the glycosyl hydrolase family 5 domain) catalyze the endohydrolysis of 1,4-β-D-glucosidic linkages in cellulose. Numerous plant proteins with this domain exist and are expressed in an organ specific manner. They are involved in the fruit ripening process, in cell elongation and plant reproduction. Modulation of the activity of these proteins, either by over- or under-expression or by mutation of the polypeptide, could be used to affect post-harvest physiology (e.g. rate of ripening) or for engineering reproductive sterility.

09595325.061502

Ref: Giorda et al. (1990) Biochemistry 29: 7264-9
Tucker et al. (1988) Plant Physiol 88: 1257-62
Shani et al. (1997) 43: 837-42
Milligan and Gasser (1995) Plant Mol Biol 28: 691-711

H. Glycosyl_hydr14

The β-amylases (family 14 of glycosyl hydrolases) catalyze the hydrolysis of 1,4-α-glucosidic linkages in polysaccharides and remove successive maltose units from the non-reducing ends of the chains. Mutants of β-amylase in Arabidopsis exhibited altered degradation of starch throughout the diurnal cycle. In addition, the mutant phenotypes indicated that these enzymes not only affect carbohydrate metabolism/catabolism, but also influence the amount of pigment stored within particular cells. Manipulation of the β-amylase genes enables control of plant pigmentation (for example, fibre pigment in cotton) as well as carbohydrate synthesis and degradation.

Ref: Zeeman et al. (1998) Plant J 15: 357-65
Hirano and Nakamura (1997) Plant Physiol 114: 5675-82
Kitamoto et al. (1988) J Bacteriol 170: 5848-54

I. Glycosyl_hydr15

Glycosyl hydrolases from family 15 (such as 1,4-Alpha-D-Glucan glucohydrolase,) catalyze the hydrolysis of terminal 1,4-linked alpha-D-glucose residues successively from the non-reducing ends of the chains resulting in the release of β–D-Glucose. In plants these proteins have been tied to the mobilization of the xyloglucan stored in the cotyledonary cell walls. Proteins such as these could be varied to affect the rate of plant growth (for example during germination), storage and/or use of glucose and other sugars by plant tissues and alteration of the properties, such as elasticity, of plant cell walls.

Ref: Crombie et al. (1998) Plant J 15: 27-38
Hata et al. (1991) Agric Biol Chem 55: 941-9

J. Glycosyl_hydr20

Members of the family 20 glycosyl hydrolases catalyze the hydrolysis of terminal non-reducing N-acetly-D-hexosamine residues in N-acetyl-β-D-hexosaminides. N-acetyl-β - glucosaminidase belongs to this family and exists in several different forms (consisting of various combinations of alpha and beta chains) depending on the organism. Family 20 glycosyl hydrolases have been implicated in lysosomal storage diseases (such as Sandhoff disease) and glycogen storage disease in humans. These types of proteins are also responsible for the hydrolysis of chitin. In plants, these proteins could be useful in controlling carbohydrate catabolism, thereby influencing the amount of sugars available for storage and/or use in other metabolic pathways. In addition, it is possible that such proteins could be used to engineer an endogenous insect protection mechanism, e.g. by secretion of a chitin-hydrolyzing composition by the plant.

Ref: Graham et al (1988) J Biol Chem 263: 16823-9
O'Dowd et al. (1988) Biochemistry 27: 5216-26

K. HMG box

The HMG box is a novel type of DNA-binding domain found in a diverse group of proteins. Numerous plant proteins contain this domain, such as the HMG1/2-like proteins. The expression of some of these HMG proteins appears to be regulated by circadian rhythms and in a light dependent manner, occurring at higher levels in roots, for example and lower levels in light-grown tissues such as cotyledons. Generally, HMG proteins are thought to influence transcription regulation. In plants, HMGs are believed to have a role in maintaining patterns of circadian-regulated expression for other genes, suggesting that these proteins could be exploited to control growth and development.

Ref: Laudet et al. (1993) Nucleic Acids Res 21: 2493-501
Zheng et al. (1993) Plant Mol Biol 23: 813-23
Grasser et al. (1993) Plant Mol Biol 23: 619-25

09555356.061600

L. IL2

Interleukin-2 (IL-2)is produced in mammals by T cells in response to antigenic or mitogenic stimulation and is crucial for proper regulation and functioning of the immune response. IL-2 is capable of stimulating B cells, monocytes, lymphokine-activated killer cells, natural killer cells and glioma cells. Plant extracts have also been shown to stimulate the immune system (for example, mistletoe therapy for human cancer). It is known that IL-2 is involved in feedback inhibition pathways that impact the inflammatory response as well as the growth inhibition of tumor reactive T cells. Plant proteins containing IL-2-like sequences are useful as immunity-based therapeutics, acting in a manner similar to IL-2 in mammals.

Ref: Heike et al. (1997) Scand J Immunol 45: 221-6
Ariel et al. (1998) J Immunol 161: 2465-72
Schink (1997) Anticancer Drugs 8 Suppl 1: S47-51

09555326.061400

M. Oxidored_FMN

NADPH dehydrogenases catalyze the reaction NADPH + acceptor = NADP(+) + reduced acceptor. One member of this family is yeast "old yellow enzyme" (OYE) and is thought to be involved in oxylipin metabolism. A second yeast family member is a protein that binds estrogen binding protein (EBP) in addition to exhibiting oxidoreductase activity. An Arabidopsis homolog to OYE has been described and estrogen binding proteins in plants have been reported. Plant proteins from this class have the potential to be used to modify lipid metabolism/catabolism. These proteins may also have use as therapeutics for breast and prostate cancer, and other abnormal growth in steroid-sensitive tissues.

Ref: Baker et al. (1998) Proc Soc Exp Biol Med 217: 317-21
Schaller and Weiler (1997) J Biol Chem 272: 28066-72
Mandani et al. (1994) PNAS USA 91: 922-6

N. Oxidored_q2

The NADH-plastoquinone oxidoreductases catalyze the reaction NADH + plastoquinone = NAD(+) + plastoquinol. In plants these reactions occur in the chloroplast and are believed to participate in a chloroplast respiratory system. Here, the NDH complex is postulated to act as a valve to remove excess reduction equivalents in the chloroplasts. Manipulation of these proteins may improve the rate or efficiency of photosynthesis.

Ref: Burrows et al. (1998) EMBO J 17: 868-76
Kofer et al (1998) Mol Gen Genet 258: 166-73
Maier et al. (1995) J Mol Biol 251: 614-28

O. PABP

Polyadenylate binding proteins bind the poly (A) tail of mRNA. Plants, as exemplified by Arabidopsis, contain numerous PABP genes that are expressed in an organ-specific manner. For example, PABP2 is functional in roots and shoots, while PABP5 is expressed predominantly in immature flowers. The PABP proteins are implicated in numerous aspects of posttranscriptional regulation including mRNA turnover and translational initiation. Control of activity of PABP proteins provides the ability to control the expression of various genes in particular organs during development.

Ref: Hilson et al (1993) Plant Physiol 103: 525-33
Belostotsky and Meagher (1993) PNAS USA 90: 6686-90

P. Parvo coat

Parvoviruses are linear single-stranded DNA viruses that are encapsulated by three capsid proteins. Plants are susceptible to infection by single stranded DNA viruses such as Maize streak virus (MSV) and various Gemini viruses. The coat proteins in these plant viruses are critical to the virus life cycle within the plant. For example, the coat protein of MSV is thought to be involved in intra- and inter-cellular movement within the plant. Engineering of proteins having similarity to parvoviral coat proteins, especially to produce proteins that interfere with maturation of the virus particle, enables the production of plants having better resistance to natural plant single-stranded DNA viruses.

09525956.071500

Ref: Liu et al. (1997) J Gen Virol 78: 1265-70
Rohde et al. (1990) Virology 176: 648-51

Q. Pkinase_C

Plant serine/threonine protein kinases possessing this domain are expressed in all tissues and are known to undergo serine-specific autophosphorylation and specifically phosphorylate two ribosomal proteins, P14 and P16. During development, these proteins predominate during high metabolic activity in growing buds, root tips, leaf margins and germinating seeds. They are thought to be involved in the control of plant growth and development. In addition, two genes encoding proteins from this family have been described that help plant cells adapt during cold or high salt stresses. Consequently, engineering Pkinase C proteins provides a way to control general growth/development of the plant as well as a means to provide endogenous protection against environmental stresses.

Ref: Zhang et al. (1994) J Biol Chem 269: 17586-92
Mizoguchi et al. (1995) FEBS Lett 358: 199-204

R. REV

The REV proteins act post-transcriptionally to relieve negative repression of GAG and ENV production in retroviruses such as Human Immounodeficiency Virus type I (HIV-1). Plants contain retrovirus-like viruses such as pararetroviruses and retrotransposons (i.e. transposons having long terminal repeats). Plant retrotransposons in particular have been used to create mutations at various loci, thereby permitting gene isolation, gene tagging and the like. Manipulation of plant REV proteins enables control of transposition frequencies of corresponding transposable elements and provides a new tool for genetic engineering of plants.

Ref: Sodroski et al. (1986) Nature 321: 412-7
Franchini et al. (1989) PNAS USA 86: 2433-7
Marquet et al. (1995) 77: 113-24

09555325.051500

U. Signal

Many plant proteins in this family contain sequences similar to those found in both components of the prokaryotic family of signal transducers known as the two-component systems. This suggests that activation may require a transfer of a phosphate group between the transmitter domain and the receiver domain. One family member in Arabidopsis appears to be involved in ethylene (a plant hormone) signal transduction. Other proteins in this family appear to be involved in the regulation of gene transcription under conditions of environmental stress. Signal proteins can be exploited to affect plant growth and development and/or control plant responses to stress conditions such as cold, nutrient availability, etc.

Ref: Chang et al. (1993) Science 262: 539-44
Nagaya et al. (1993) Gene 131: 119-124
Gottfert et al. (1990) PNAS USA 87: 2680-4

09525256.061500

V. vMSA

vMSA proteins are major surface antigens presenting on the envelope of various retroviruses. Surface antigens of retroviruses are often involved in tropism of the virus. Plants contain retrovirus-like viruses such as pararetroviruses and retrotransposons (i.e. transposons having long terminal repeats). Plant retrotransposons in particular have been used to create mutants at various loci, thereby permitting gene isolation, gene tagging and the like. Manipulation of plant vMSA proteins enables control of tropism of plant retroviruses that might be used for genetic engineering tools, thus enabling targeting of the virus to particular species and/or tissues of plants.

Ref: Okamoto et al. (1988) J Gen Virol 69: 2575-83
Grandbastien et al. (1989) Nature 337: 376-80
Wright and Voytas (1998) Genetics 149: 703-15

W. zf-CCCH

This family of proteins is defined by having two CX(8)CX(5)CX(3)H-type zinc finger domains. These proteins cover a broad range of functions. For example, the COP1 protein acts as a repressor of photomorphogenesis in darkness; light stimuli abolish this suppressive action. In addition, COP1 protein can function as a negative transcriptional regulator capable of direct interaction with components of the G-protein signaling pathway. As a second example, a zf-CCCH protein identified in Arabidopsis appears to be involved in the resistance to DNA damage induced by UV light and chemical DNA-damaging agents. Overexpression of this class of proteins permits production of plants that are better suited to adverse environments. Manipulation of expression of zf-CCCH proteins functioning as transcriptional regulators, such as COP1, enables manipulation of some signal transduction pathways.

Ref: Pang et al. (1993) Nucleic Acids Res 21: 1647-53
Deng et al. (1992) Cell 71: 791-801

X. zf-RanBP

Proteins falling within this category contain many X-X-F-G and X-F-X-F-G repeats, and may contain RANBP1-like or PPIase domains. Plant proteins having domains similar to these include PAS1 and GMSTI. PAS1 has been shown to have dramatic developmental affects that appear to be correlated with both cell division and cell wall elongation. GMSTI has high identity to the yeast STI stress-inducible gene and has been shown to be heat inducible. Proteins such as these may be useful for controlling growth and form of development.

Ref: Vittorioso et al. (1998) Mol Cell Biol 18: 3034-43
Hernandez Torres et al. (1995) 27: 1221-6

Y. Peptidase M48.

Proteins belonging to this peptidase family are metalloproteases that bind zinc as a cofactor and are located in the membranes of the endoplasmic reticulum. They function in NH_2-terminal proteolytic processing, as shown for the yeast STE24 gene product. This gene is required for the correct processing of α-factor, a yeast pheromone. Family M48 peptidases also appear to be required for some prenylation reactions, mediating COOH-terminal CAAX processing. Prenylation reactions are believed to be involved in the regulation of protein-protein and protein-membrane interactions. As an example, RAS GTPase activity is regulated in part by localization to the inner side of the plasma membrane upon prenylation. In plants, proteins from this family could be involved in pollen-stigma interactions such as those mediating self-pollenation vs. outcrossing, or could be members of several secondary metabolism pathways.

Ref: Fujimura-Kamada et al. (1997) J Cell Biol. 136: 271-85. Tam et al. (1998) J Cell Biol. 142: 635-49.

Z. DNA Pol Viral N

The DNA pol Viral N domain is located at the N-terminal region of DNA polymerase isolated from several retroid viruses such as the Cauliflower Mosaic Virus. The domain motif has also been found in numerous other species from humans to cyanobacteria. In these organisms, this motif seems to be associated with two types of sequences; retrotransposons and mitochondrial genes. In the mitochondrial sequences this domain is potentially involved in the self-splicing conducted by group II introns. Various manipulations of this gene in plants allows control of the numerous retrotransposons endogenous to plant genomes or allows engineering of mitochondrial function, especially to increase efficiency of energy utilization by cells.

REF: Chapdelaine and Bonen (1991) Cell 65: 465-72
Ferat and Miche (1993) Nature 364: 358-61
Wilson et al. (1994) 368: 32-8
Cambareri et al. (1994) 242: 658-65
Gaardner et al. (1981) NAR 9: 2871-2888
Cummings et al. (1990) Curr Genet 17: 375-402
Hattori et al. (1986) Nature 321: 625-8

Aa. Calpain_inhib

This domain is found in calpastatin, an inhibitor protein specific for calpain. Calpain is a non-lysosomal calcium-dependent intracellular protease that appears to be involved in the dynamic changes of the cytoskeleton, especially actin-related structures, during early *Drosophila* embryogenesis [1]. Calpastatins co-exist in cells with calpains and the subcellular distribution of calpastatin is thought to be important to calpain regulation [2]. In plants calpains and calpastatins could be involved in embryogenesis and non-embryogenic organ reiteration. Mutations occurring in calpain inhibitor repeat domains would produce developmental abnormalities such as abnormal leaf, root or flower development.

Refs

1 Emori Y and Saigo K (1994) J Biol Chem 269: 25137-42.

2 Mellgren RL, Lane RD, Mericle MT (1989) Biochim Biophys Acta 999: 71-77.

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Ab. chorismate_bind

Chorismate binding domains are present in plant anthranilate synthase (AS) genes. AS genes catalyze the first step in the biosynthesis of tryptophan by converting chorismate and L-glutamine to anthranilate, pyruvate and L-glutamate. Some of these genes are involved in feedback inhibition by tryptophan [1] while some are feedback insensitive [2]. In Arabidopsis, two AS genes have overlapping, but different distributions. One of these AS genes is induced by wounding and bacterial pathogen infiltration [1]. Mutations in the chorismate binding domain would affect the production of tryptophan and could influence the plant's defense system. AS gene products can be used for *in vitro* synthesis of tryptophan and tryptophan derivatives.

Refs

1 Niyogi KK, Fink GR (1992) Plant Cell 4: 721-33.

2 Song HS, Brotherton JE, Gonzales RA, Wilholm JM (1998) Plant Physiol 117:533-43.

Ac. late_protein_L2

Papillomaviruses are encapsulated double stranded DNA viruses. Plants are susceptible to infection by double stranded DNA viruses such as Cauliflower Mosaic virus (CaMV). The coat proteins in these plant viruses are critical to the virus life cycle within the plant. For example, the coat protein of CaMV is thought to be involved in intra- and inter-cellular movement within the plant [1]. Engineering of proteins having similarity to papillomavirus coat proteins may enable the production of plants having better resistance to natural plant double stranded DNA viruses.

Refs

1 Thompson SR, Melcher U (1993) J Gen Virol 74: 1141-8.

Ad. Peptidase_M41

Proteins belonging to this peptidase family are metalloproteases that bind zinc as a cofactor and are integral membrane proteins. They seem to be involved in the degradation of carboxy-terminal-tagged cytoplasmic proteins. In plants, these proteins are located in the thylakoid membranes of the chloroplasts, their expression is light regulated and they are thought to be involved in degradation of soluble stromal proteins and turn-over of thylkoid proteins [1]. Manipulation of expression and structure of these proteins would have effects on the efficiency of photosynthesis and the development of chloroplasts.

09555256.061500

Refs

1 Lindahl M, Tabak s, Cseke L, Pichersky E, Andersson B, Adam Z (1996) J Biol Chem 271: 29329-34.

Ae. UPF0051

There is some evidence that, in plants, proteins in this family are involved in ATP synthesis in chloroplasts [1, 2]. Mutations in these proteins or altering their expression would affect the efficiency of photosynthesis and energy production.

Refs

1 Kostrzewa M, Zetsche K (1992) J Mol Biol 227: 961-70.

2 Kostrzewa M, Zetsche K (1993) Plant Mol Biol 23: 67-76

Af. E7

Papillomaviruses are encapsulated double stranded DNA viruses. The Papillomavirus early protein 7 (E7) is known as a potent immortalizing and transforming agent. Transformation by E7 is thought to be mediated by the physical association of E7 with cellular proteins regulating entry into the cell cycle [1]. The result is entry into the cell cycle and suppression of terminal differentiation in mammalian cells. Thus, engineering of proteins having similarity to papillomavirus E7 protein enables the production of plants having altered cellular proliferation characteristics and possibly altered morphology. For example, overexpression of E7-like proteins would be expected to result in proliferation of cells of the tissue in which the E7 protein is expressed, perhaps with suppression of differentiation events. Thus, for example, overexpression of E7-like proteins in meristem cells can result in taller plants and suppression of leafing and/or flowering.

Refs

1 Zwerschke W, Jansen-Durr P Adv Cancer Res 2000;78:1-29

09953625.071902

Ag. Peptidase U7

This protein is known to be an integral membrane protein in the cyanobacterium Synechocystis where it functions to digest cleaved signal peptides [1]. This activity is necessary to maintain proper secretion of mature proteins across the membrane. In higher plants this protein may be present in the plastid or chloroplast membranes where it would function by enabling protein movement into and out of the chloroplasts. Mutations in this protein would be expected to affect the development of plastids, including chloroplasts, or alter the energy transfer system within the chloroplasts, thereby affecting growth and development.

Refs

1 Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakazaki N, Naruo K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yamada M, Yasuda M, Tabata S (1996) DNA Res 3:109-36.

Ah. 5’-3’ Exonuclease

The 5'-3' exonuclease domain is one found in bacterial DNA polymerases I and in yeast DNA repair enzymes such as Exonuclease I. Yeast Exo I is involved in mitotic recombination and also includes a domain that interacts with the mismatch repair protein MSH2. The 5'-3' exonuclease domain is also present in XPG DNA repair enzymes in humans and in yeast RAD9 protein. Defects in XPG proteins result in Xeroderma Pigmentosum. Thus defects in 5'-3' exonuclease domain-containing proteins in plants are expected to lead to defects in DNA repair and corresponding high spontaneous and inducible mutation rates. Consensus sequence:

IMKKKLLLVDGSSLAFRAFFALPPLTNSAGEPTNAVYGFLKMLIKLIEQEQPTHIAVV
FDAKAKTFRHELYEGYKAGRAP
TPDELREQIPLIKELLDALGIPLLEVAGYEADDVIGTLAKLAEKEGYEVLIVTGDRDLL
QLVSDHVTVIITKKGIAEFTL
FTPEAVIEKYGLTPEQIIDYKALMGDSSDNIPGVKGIGEKTAAKLLQEYGSLEGIYANL
DKLKGKKLREKLLAHKEDAKL
SRDLATIKTDVPLDLTLDDLRLPDPDRDALDLLFDE

Ref:

Fiorentini P. et al. RT. Mol. Cell. Biol. 17:2764-2773(1997).

Tishkoff et al. Cancer Res. 0:0-0(1998).

Macinnes M.A. et al. Mol. Cell. Biol. 13:6393-6402(1993).

AA. Activities of Polypeptides Comprising Signal Peptides

Polypeptides comprising signal peptides are a family of proteins that are typically targeted to (1) a particular organelle or intracellular compartment, (2) interact with a particular molecule or (3) for secretion outside of a host cell. Example of polypeptides comprising signal peptides include, without limitation, secreted proteins, soluble proteins, receptors, proteins retained in the ER, etc.

These proteins comprising signal peptides are useful to modulate ligand-receptor interactions, cell-to-cell communication, signal transduction, intracellular communication, and activities and/or chemical cascades that take part in an organism outside or within of any particular cell.

One class of such proteins are soluble proteins which are transported out of the cell. These proteins can act as ligands that bind to receptor to trigger signal transduction or to permit communication between cells.

Another class is receptor proteins which also comprise a retention domain that lodges the receptor protein in the membrane when the cell transports the receptor to the surface of the cell. Like the soluble ligands, receptors can also modulate signal transduction and communication between cells.

In addition the signal peptide itself can serve as a ligand for some receptors. An example is the interaction of the ER targeting signal peptide with the signal recognition particle (SRP). Here, the SRP binds to the signal peptide, halting translation, and the resulting SRP complex then binds to docking proteins located on the surface of the ER, prompting transfer of the protein into the ER.

A description of signal peptide residue composition is described below in Subsection IV.C.1.

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III. Methods of Modulating Polypeptide Production

It is contemplated that polynucleotides of the invention can be incorporated into a host cell or in-vitro system to modulate polypeptide production. For instance, the SDFs prepared as described herein can be used to prepare expression cassettes useful in a number of techniques for suppressing or enhancing expression.

An example are polynucleotides comprising sequences to be transcribed, such as coding sequences, of the present invention can be inserted into nucleic acid constructs to modulate polypeptide production. Typically, such sequences to be transcribed are heterologous to at least one element of the nucleic acid construct to generate a chimeric gene or construct.

Another example of useful polynucleotides are nucleic acid molecules comprising regulatory sequences of the present invention. Chimeric genes or constructs can be generated when the regulatory sequences of the invention linked to heterologous sequences in a vector construct. Within the scope of invention are such chimeric gene and/or constructs.

Also within the scope of the invention are nucleic acid molecules, whereof at least a part or fragment of these DNA molecules are presented in TABLE 1 of the present application, and wherein the coding sequence is under the control of its own promoter and/or its own regulatory elements. Such molecules are useful for transforming the genome of a host cell or an organism regenerated from said host cell for modulating polypeptide production.

Additionally, a vector capable of producing the oligonucleotide can be inserted into the host cell to deliver the oligonucleotide.

More detailed description of components to be included in vector constructs are described both above and below.

Whether the chimeric vectors or native nucleic acids are utilized, such polynucleotides can be incorporated into a host cell to modulate polypeptide production. Native genes and/or nucleic acid molecules can be effective when exogenous to the host cell.

Methods of modulating polypeptide expression includes, without limitation:

Suppression methods, such as

- Antisense
- Ribozymes
- Co-suppression
- Insertion of Sequences into the Gene to be Modulated

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Regulatory Sequence Modulation.

as well as Methods for Enhancing Production, such as
Insertion of Exogenous Sequences; and
Regulatory Sequence Modulation.

III.A. Suppression

Expression cassettes of the invention can be used to suppress expression of endogenous genes which comprise the SDF sequence. Inhibiting expression can be useful, for instance, to tailor the ripening characteristics of a fruit (Oeller et al., *Science* 254:437 (1991)) or to influence seed size_(WO98/07842) or to provoke cell ablation (Mariani et al., Nature 357: 384-387 (1992).

As described above, a number of methods can be used to inhibit gene expression in plants, such as antisense, ribozyme, introduction of exogenous genes into a host cell, insertion of a polynucleotide sequence into the coding sequence and/or the promoter of the endogenous gene of interest, and the like.

III.A.1. Antisense

An expression cassette as described above can be transformed into host cell or plant to produce an antisense strand of RNA. For plant cells, antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, *see*, e.g., Sheehy et al., *Proc. Nat. Acad. Sci.* USA, 85:8805 (1988), and Hiatt et al., U.S. Patent No. 4,801,340.

III.A.2. Ribozymes

Similarly, ribozyme constructs can be transformed into a plant to cleave mRNA and down-regulate translation.

III.A.3. Co-Suppression

Another method of suppression is by introducing an exogenous copy of the gene to be suppressed. Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter has been shown to prevent the accumulation of mRNA. A detailed description of this method is described above.

III.A.4. Insertion of Sequences into the Gene to be Modulated

Yet another means of suppressing gene expression is to insert a polynucleotide into the gene of interest to disrupt transcription or translation of the gene.

Homologous recombination could be used to target a polynucleotide insert to a gene using the Cre-Lox system (A.C. Vergunst et al., *Nucleic Acids Res.* 26:2729 (1998), A.C. Vergunst et al., *Plant Mol. Biol.* 38:393 (1998), H. Albert et al., *Plant J.* 7:649 (1995)).

In addition, random insertion of polynucleotides into a host cell genome can also be used to disrupt the gene of interest. Azpiroz-Leehan et al., *Trends in Genetics* 13:152 (1997). In this method, screening for clones from a library containing random insertions is preferred for identifying those that have polynucleotides inserted into the gene of interest. Such screening can be performed using probes and/or primers described above based on sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto. The screening can also be performed by selecting clones or any transgenic plants having a desired phenotype.

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III.A.5. Regulatory SequenceModulation

The SDFs described in Table 1, and fragments thereof are examples of nucleotides of the invention that contain regulatory sequences that can be used to suppress or inactivate transcription and/or translation from a gene of interest as discussed in I.C.5.

III.A.6. Genes Comprising Dominant-Negative Mutations

When suppression of production of the endogenous, native protein is desired it is often helpful to express a gene comprising a dominant negative mutation. Production of protein variants produced from genes comprising dominant negative mutations is a useful tool for research Genes comprising dominant negative mutations can produce a variant polypeptide which is capable of competing with the native polypeptide, but which does not produce the native result. Consequently, over expression of genes comprising these mutations can titrate out an undesired activity of the native protein. For example, The product from a gene comprising a dominant negative mutation of a receptor can be used to constitutively activate or suppress a signal transduction cascade, allowing examination of the phenotype and thus the trait(s) controlled by that receptor and pathway. Alternatively, the protein arising from the gene comprising a dominant-negative mutation can be an inactive enzyme still capable of binding to the same substrate as the native protein and therefore competes with such native protein.

Products from genes comprising dominant-negative mutations can also act upon the native protein itself to prevent activity. For example, the native protein may be active only as a homo-multimer or as one subunit of a hetero-multimer. Incorporation of an inactive subunit into the multimer with native subunit(s) can inhibit activity.

Thus, gene function can be modulated in host cells of interest by insertion into these cells vector constructs comprising a gene comprising a dominant-negative mutation.

III.B. Enhanced Expression

Enhanced expression of a gene of interest in a host cell can be accomplished by either (1) insertion of an exogenous gene; or (2) promoter modulation.

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III.B.1. Insertion of an Exogenous Gene

Insertion of an expression construct encoding an exogenous gene can boost the number of gene copies expressed in a host cell.

Such expression constructs can comprise genes that either encode the native protein that is of interest or that encode a variant that exhibits enhanced activity as compared to the native protein. Such genes encoding proteins of interest can be constructed from the sequences from TABLE 1, fragments thereof, and substantially similar sequence thereto.

Such an exogenous gene can include either a constitutive promoter permitting expression in any cell in a host organism or a promoter that directs transcription only in particular cells or times during a host cell life cycle or in response to environmental stimuli.

III.B.2. Regulatory Sequence Modulation

The SDFs of Table 1, and fragments thereof, contain regulatory sequences that can be used to enhance expression of a gene of interest. For example, some of these sequences contain useful enhancer elements. In some cases, duplication of enhancer elements or insertion of exogenous enhancer elements will increase expression of a desired gene from a particular promoter. As other examples, all ll promoters require binding of a regulatory protein to be activated, while some promoters may need a protein that signals a promoter binding protein to expose a polymerase binding site. In either case, over-production of such proteins can be used to enhance expression of a gene of interest by increasing the activation time of the promoter.

Such regulatory proteins are encoded by some of the sequences in TABLE 1, fragments thereof, and substantially similar sequences thereto.

Coding sequences for these proteins can be constructed as described above.

IV. Gene Constructs and Vector Construction

To use isolated SDFs of the present invention or a combination of them or parts and/or mutants and/or fusions of said SDFs in the above techniques, recombinant DNA vectors which comprise said SDFs and are suitable for transformation of cells, such as plant cells, are usually prepared. The SDF construct can be made using standard recombinant DNA techniques (Sambrook et al. 1989) and can be introduced to the species of interest by *Agrobacterium*-mediated transformation or by other means of transformation (*e.g.*, particle gun bombardment) as referenced below.

The vector backbone can be any of those typical in the art such as plasmids, viruses, artificial chromosomes, BACs, YACs and PACs and vectors of the sort described by

(a) **BAC:** Shizuya et al., Proc. Natl. Acad. Sci. USA 89: 8794-8797 (1992); Hamilton et al., Proc. Natl. Acad. Sci. USA 93: 9975-9979 (1996);

(b) **YAC:** Burke et al., Science 236:806-812 (1987);.

(c) **PAC:** Sternberg N. et al., Proc Natl Acad Sci U S A. Jan;87(1):103-7 (1990);

(d) **Bacteria-Yeast Shuttle Vectors:** Bradshaw et al., Nucl Acids Res 23: 4850-4856 (1995);

(e) **Lambda Phage Vectors**: Replacement Vector, e.g., Frischauf et al., J. Mol Biol 170: 827-842 (1983); or Insertion vector, e.g., Huynh et al., In: Glover NM (ed) DNA Cloning: A practical Approach, Vol.1 Oxford: IRL Press (1985);

(f) **T-DNA gene fusion vectors :**Walden et al., Mol Cell Biol 1: 175-194 (1990); and

(g) **Plasmid vectors:** Sambrook et al., infra.

Typically, a vector will comprise the exogenous gene, which in its turn comprises an SDF of the present invention to be introduced into the genome of a host cell, and which gene may be an antisense construct, a ribozyme construct chimeraplast, or a coding sequence with any desired transcriptional and/or translational regulatory sequences, such as promoters, UTRs,

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and 3' end termination sequences. Vectors of the invention can also include origins of replication, scaffold attachment regions (SARs), markers, homologous sequences, introns, etc.

A DNA sequence coding for the desired polypeptide, for example a cDNA sequence encoding a full length protein, will preferably be combined with transcriptional and translational initiation regulatory sequences which will direct the transcription of the sequence from the gene in the intended tissues of the transformed plant.

For example, for over-expression, a plant promoter fragment may be employed that will direct transcription of the gene in all tissues of a regenerated plant. Alternatively, the plant promoter may direct transcription of an SDF of the invention in a specific tissue (tissue-specific promoters) or may be otherwise under more precise environmental control (inducible promoters).

If proper polypeptide productionis desired, a polyadenylation region at the 3'-end of the coding region is typically included. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA.

The vector comprising the sequences from genes or SDF or the invention may comprise a marker gene that confers a selectable phenotype on plant cells. The vector can include promoter and coding sequence, for instance. For example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosulfuron or phosphinotricin.

IV.A. Coding Sequences

Generally, the sequence in the transformation vector and to be introduced into the genome of the host cell does not need to be absolutely identical to an SDF of the present invention. Also, it is not necessary for it to be full length, relative to either the primary transcription product or fully processed mRNA. Furthermore, the introduced sequence need not have the same intron or exon pattern as a native gene. Also, heterologous non-coding segments can be incorporated into the coding sequence without changing the desired amino acid sequence of the polypeptide to be produced.

IV.B. Promoters

As explained above, introducing an exogenous SDF from the same species or an orthologous SDF from another species can modulate the expression of a native gene corresponding to that SDF of interest. Such an SDF construct can be under the control of either a constitutive promoter or a highly regulated inducible promoter (*e.g.*, a copper inducible promoter). The promoter of interest can initially be either endogenous or heterologous to the species in question. When re-introduced into the genome of said species, such promoter becomes exogenous to said species. Over-expression of an SDF transgene can lead to co-suppression of the homologous endogeneous sequence thereby creating some alterations in the phenotypes of the transformed species as demonstrated by similar analysis of the chalcone synthase gene (Napoli et al., *Plant Cell* 2:279 (1990) and van der Krol et al., *Plant Cell* 2:291 (1990)). If an SDF is found to encode a protein with desirable characteristics, its over-production can be controlled so that its accumulation can be manipulated in an organ- or tissue-specific manner utilizing a promoter having such specificity.

Likewise, if the promoter of an SDF (or an SDF that includes a promoter) is found to be tissue-specific or developmentally regulated, such a promoter can be utilized to drive or facilitate the transcription of a specific gene of interest (*e.g.*, seed storage protein or root-specific protein). Thus, the level of accumulation of a particular protein can be manipulated or its spatial localization in an organ- or tissue- specific manner can be altered.

IV. C Signal Peptides

SDFs of the present invention containing signal peptides are indicated in Table 1. In some cases it may be desirable for the protein encoded by an introduced exogenous or orthologous SDF to be targeted (1) to a particular organelle intracellular compartment, (2) to interact with a particular molecule such as a membrane molecule or (3) for secretion outside of the cell harboring the introduced SDF. This will be accomplished using a signal peptide.

Signal peptides direct protein targeting, are involved in ligand-receptor interactions and act in cell to cell communication. Many proteins, especially soluble proteins, contain a signal peptide that targets the protein to one of several different intracellular compartments. In plants, these compartments include, but are not limited to, the endoplasmic reticulum (ER), mitochondria, plastids (such as chloroplasts), the vacuole, the Golgi apparatus, protein storage vessicles (PSV) and, in general, membranes. Some signal peptide sequences are conserved, such as the Asn-Pro-Ile-Arg amino acid motif found in the N-terminal propeptide signal that targets proteins to the vacuole (Marty (1999) *The Plant Cell* 11: 587-599). Other signal peptides do not have a consensus sequence *per se*, but are largely composed of hydrophobic amino acids, such as those signal peptides targeting proteins to the ER (Vitale and Denecke (1999) *The Plant Cell* 11: 615-628). Still others do not appear to contain either a consensus sequence or an identified common secondary sequence, for instance the chloroplast stromal targeting signal peptides (Keegstra and Cline (1999) *The Plant Cell* 11: 557-570). Furthermore, some targeting peptides are bipartite, directing proteins first to an organelle and then to a membrane within the organelle (e.g. within the thylakoid lumen of the chloroplast; see Keegstra and Cline (1999) *The Plant Cell* 11: 557-570). In addition to the diversity in sequence and secondary structure, placement of the signal peptide is also varied. Proteins destined for the vacuole, for example, have targeting signal peptides found at the N-terminus, at the C-terminus and at a surface location in mature, folded proteins. Signal peptides also serve as ligands for some receptors.

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These characteristics of signal proteins can be used to more tightly control the phenotypic expression of introduced SDFs. In particular, associating the appropriate signal sequence with a specific SDF can allow sequestering of the protein in specific organelles (plastids, as an example), secretion outside of the cell, targeting interaction with particular receptors, etc. Hence, the inclusion of signal proteins in constructs involving the SDFs of the invention increases the range of manipulation of SDF phenotypic expression. The nucleotide sequence of the signal peptide can be isolated from characterized genes using common molecular biological techniques or can be synthesized in vitro.

In addition, the native signal peptide sequences, both amino acid and nucleotide, described in Table 1 can be used to modulate polypeptide transport. Further variants of the native signal peptides described in Table 1 are contemplated. Insertions, deletions, or substitutions can be made. Such variants will retain at least one of the functions of the native signal peptide as well as exhibiting some degree of sequence identity to the native sequence.

Also, fragments of the signal peptides of the invention are useful and can be fused with other signal peptides of interest to modulate transport of a polypeptide.

V. Transformation Techniques

A wide range of techniques for inserting exogenous polynucleotides are known for a number of host cells, including, without limitation, bacterial, yeast, mammalian, insect and plant cells.

Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. *See, e.g.* Weising et al., *Ann. Rev. Genet.* 22:421 (1988); and Christou, Euphytica, v. 85, n.1-3:13-27, (1995).

DNA constructs of the invention may be introduced into the genome of the desired plant host by a variety of conventional techniques. For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the DNA constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria (McCormac et al., *Mol. Biotechnol.* 8:199 (1997); Hamilton, *Gene* 200:107 (1997)); Salomon et al. *EMBO J.* 3:141 (1984); Herrera-Estrella et al. *EMBO J.* 2:987 (1983).

Microinjection techniques are known in the art and well described in the scientific and patent literature. The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski et al. *EMBO J.* 3:2717 (1984). Electroporation techniques are described in Fromm et al. *Proc. Natl Acad. Sci. USA* 82:5824 (1985). Ballistic transformation techniques are described in Klein et al. *Nature* 327:773 (1987). *Agrobacterium tumefaciens*-mediated transformation techniques, including disarming and use of binary or co-integrate vectors, are well described in the scientific literature. See, for example Hamilton, *CM.*, *Gene* 200:107 (1997); Müller et al. *Mol. Gen. Genet.* 207:171 (1987); Komari et al. *Plant J.* 10:165 (1996); Venkateswarlu et al. *Biotechnology* 9:1103 (1991) and Gleave, *AP.*, *Plant Mol. Biol.* 20:1203 (1992); Graves and Goldman, *Plant Mol. Biol.* 7:34 (1986) and Gould et al., *Plant Physiology* 95:426 (1991).

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant that possesses the transformed genotype and thus the desired phenotype such as seedlessness. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., *Protoplasts Isolation and Culture* in "Handbook of Plant Cell Culture," pp. 124-176, MacMillan Publishing Company, New York, 1983; and Binding, *Regeneration of Plants, Plant Protoplasts,* pp. 21-73, CRC Press, Boca Raton, 1988. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al. *Ann. Rev. of Plant Phys.* 38:467 (1987). Regeneration of monocots (rice) is described by Hosoyama et al. (*Biosci. Biotechnol. Biochem.* 58:1500 (1994)) and by Ghosh et al. (*J. Biotechnol.* 32:1 (1994)). The nucleic acids of the invention can be used to confer desired traits on essentially any plant.

Thus, the invention has use over a broad range of plants, including species from the genera *Anacardium, Arachis, Asparagus, Atropa, Avena, Brassica, Citrus, Citrullus, Capsicum, Carthamus, Cocos, Coffea, Cucumis, Cucurbita, Daucus, Elaeis, Fragaria, Glycine, Gossypium, Helianthus, Heterocallis, Hordeum, Hyoscyamus, Lactuca, Linum, Lolium,Lupinus, Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Olea, Oryza, Panieum, Pannesetum, Persea, Phaseolus, Pistachia, Pisum, Pyrus, Prunus, Raphanus, Ricinus, Secale, Senecio, Sinapis, Solanum, Sorghum, Theobromus, Trigonella, Triticum, Vicia, Vitis, Vigna, and, Zea.*

One of skill will recognize that after the expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

The particular sequences of SDFs identified are provided in the attached TABLE 1. One of ordinary skill in the art, having this data, can obtain cloned DNA fragments, synthetic DNA fragments or polypeptides constituting desired sequences by recombinant methodology known in the art or described herein.

EXAMPLES

The invention is illustrated by way of the following examples. The invention is not limited by these examples as the scope of the invention is defined solely by the claims following.

EXAMPLE 1: cDNA PREPARATION

A number of the nucleotide sequences disclosed in TABLE 1 herein as representative of the SDFs of the invention can be obtained by sequencing genomic DNA (gDNA) and/or cDNA from corn plants grown from HYBRID SEED # 35A19, purchased from Pioneer Hi-Bred International, Inc., Supply Management, P.O. Box 256, Johnston, Iowa 50131-0256.

A number of the nucleotide sequences disclosed in TABLE 1 herein as representative of the SDFs of the invention can also be obtained by sequencing genomic DNA from *Arabidopsis thaliana*, Wassilewskija ecotype or by sequencing cDNA obtained from mRNA from such plants as described below. This is a true breeding strain. Seeds of the plant are available from the Arabidopsis Biological Resource Center at the Ohio State University, under the accession number CS2360. Seeds of this plant were deposited under the terms and conditions of the Budapest Treaty at the American Type Culture Collection, Manassas, VA on August 31, 1999, and were assigned ATCC No. PTA-595.

Other methods for cloning full-length cDNA are described, for example, by Seki et al., *Plant Journal* 15:707-720 (1998) "High-efficiency cloning of Arabidopsis full-length cDNA by biotinylated Cap trapper"; Maruyama et al., *Gene* 138:171 (1994) "Oligo-capping a simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides"; and WO 96/34981.

Tissues were, or each organ was, individually pulverized and frozen in liquid nitrogen. Next, the samples were homogenized in the presence of detergents and then centrifuged. The debris and nuclei were removed from the sample and more detergents were added to the sample. The sample was centrifuged and the debris was removed. Then the sample was applied to a 2M sucrose cushion to isolate polysomes. The RNA was isolated by treatment with detergents and proteinase K followed by ethanol precipitation and centrifugation. The polysomal RNA from the different tissues was pooled according to the following mass ratios: 15/15/1 for male inflorescences, female inflorescences and root, respectively. The pooled material was then used for cDNA synthesis by the methods described below.

Starting material for cDNA synthesis for the exemplary corn cDNA clones with sequences presented in TABLE 1 was poly(A)-containing polysomal mRNAs from inflorescences and root tissues of corn plants grown from HYBRID SEED # 35A19. Male inflorescences and female (pre-and post-fertilization) inflorescences were isolated at various stages of development. Selection for poly(A) containing polysomal RNA was done using oligo d(T) cellulose columns, as described by Cox and Goldberg, "Plant Molecular Biology: A Practical Approach", pp. 1-35, Shaw ed., c. 1988 by IRL, Oxford. The quality and the integrity of the polyA+ RNAs were evaluated.

Starting material for cDNA synthesis for the exemplary *Arabidopsis* cDNA clones with sequences presented in TABLE 1 was polysomal RNA isolated from the top-most inflorescence tissues of *Arabidopsis thaliana* Wassilewskija (Ws.) and from roots of *Arabidopsis thaliana* Landsberg erecta (L. er.), also obtained from the Arabidopsis Biological Resource Center. Nine parts inflorescence to every part root was used, as measured by wet mass. Tissue was pulverized and exposed to liquid nitrogen. Next, the sample was homogenized in the presence of detergents and then centrifuged. The debris and nuclei were removed from the sample and more detergents were added to the sample. The sample was centrifuged and the debris was removed and the sample was applied to a 2M sucrose cushion to isolate polysomal RNA. Cox et al., "Plant Molecular Biology: A Practical Approach", pp. 1-35, Shaw ed., c. 1988 by IRL, Oxford. The polysomal RNA was used for cDNA synthesis by the methods described below. Polysomal mRNA was then isolated as described above for corn cDNA. The quality of the RNA was assessed electrophoretically.

Following preparation of the mRNAs from various tissues as described above, selection of mRNA with intact 5' ends and specific attachment of an oligonucleotide tag to the 5' end of such mRNA was performed using either a chemical or enzymatic approach. Both techniques take advantage of the presence of the "cap" structure, which characterizes the 5' end of most intact mRNAs and which comprises a guanosine generally methylated once, at the 7 position.

The chemical modification approach involves the optional elimination of the 2', 3'-cis diol of the 3' terminal ribose, the oxidation of the 2', 3'-cis diol of the ribose linked to the cap of the 5' ends of the mRNAs into a dialdehyde, and the coupling of the such obtained dialdehyde to a derivatized oligonucleotide tag. Further detail regarding the chemical approaches for obtaining mRNAs having intact 5' ends are disclosed in International Application No. WO96/34981 published November 7, 1996.

The enzymatic approach for ligating the oligonucleotide tag to the intact 5' ends of mRNAs involves the removal of the phosphate groups present on the 5' ends of uncapped incomplete mRNAs, the subsequent decapping of mRNAs having intact 5' ends and the ligation of the phosphate present at the 5' end of the decapped mRNA to an oligonucleotide tag. Further detail regarding the enzymatic approaches for obtaining mRNAs having intact 5' ends are disclosed in Dumas Milne Edwards J.B. (Doctoral Thesis of Paris VI University, Le clonage des ADNc complets: difficultés et perspectives nouvelles. Apports pour l'étude de la régulation de l'expression de la tryptophane hydroxylase de rat, 20 Dec. 1993), EP0 625572 and Kato *et al., Gene* 150:243-250 (1994).

In both the chemical and the enzymatic approach, the oligonucleotide tag has a restriction enzyme site (e.g. an EcoRI site) therein to facilitate later cloning procedures. Following attachment of the oligonucleotide tag to the mRNA, the integrity of the mRNA is examined by performing a Northern blot using a probe complementary to the oligonucleotide tag.

For the mRNAs joined to oligonucleotide tags using either the chemical or the enzymatic method, first strand cDNA synthesis is performed using an oligo-dT primer with reverse transcriptase. This oligo-dT primer can contain an internal tag of at least 4 nucleotides, which can be different from one mRNA preparation to another. Methylated dCTP is used for cDNA first strand synthesis to protect the internal EcoRI sites from digestion during subsequent steps. The first strand cDNA is precipitated using isopropanol after removal of RNA by alkaline hydrolysis to eliminate residual primers.

Second strand cDNA synthesis is conducted using a DNA polymerase, such as Klenow fragment and a primer corresponding to the 5' end of the ligated oligonucleotide. The primer is typically 20-25 bases in length. Methylated dCTP is used for second strand synthesis in order to protect internal EcoRI sites in the cDNA from digestion during the cloning process.

Following second strand synthesis, the full-length cDNAs are cloned into a phagemid vector, such as pBlueScript™ (Stratagene). The ends of the full-length cDNAs are blunted with T4 DNA polymerase (Biolabs) and the cDNA is digested with EcoRI. Since methylated dCTP is used during cDNA synthesis, the EcoRI site present in the tag is the only hemi-methylated site; hence the only site susceptible to EcoRI digestion. In some instances, to facilitate subcloning, an Hind III adapter is added to the 3' end of full-length cDNAs.

The full-length cDNAs are then size fractionated using either exclusion chromatography (AcA, Biosepra) or electrophoretic separation which yields 3 to 6 different fractions. The full-length cDNAs are then directionally cloned either into pBlueScript™ using either the EcoRI and SmaI restriction sites or, when the Hind III adapter is present in the full-length cDNAs, the EcoRI and Hind III restriction sites. The ligation mixture is transformed, preferably by electroporation, into bacteria, which are then propagated under appropriate antibiotic selection.

Clones containing the oligonucleotide tag attached to full-length cDNAs are selected as follows.

The plasmid cDNA libraries made as described above are purified (e.g. by a column available from Qiagen). A positive selection of the tagged clones is performed as follows. Briefly, in this selection procedure, the plasmid DNA is converted to single stranded DNA using phage F1 gene II endonuclease in combination with an exonuclease (Chang et al., *Gene* 127:95 (1993)) such as exonuclease III or T7 gene 6 exonuclease. The resulting single stranded DNA is then purified using paramagnetic beads as described by Fry et al., *Biotechniques* 13: 124 (1992). Here the single stranded DNA is hybridized with a biotinylated oligonucleotide having a sequence corresponding to the 3' end of the oligonucleotide tag. Preferably, the primer has a length of 20-25 bases. Clones including a sequence complementary to the biotinylated oligonucleotide are selected by incubation with streptavidin coated magnetic beads followed by magnetic capture. After capture of the positive clones, the plasmid DNA is released from the magnetic beads and converted into double stranded DNA using a DNA polymerase such as ThermoSequenase™ (obtained from Amersham Pharmacia Biotech). Alternatively, protocols such as the Gene Trapper™ kit (Gibco BRL) can be used. The double stranded DNA is then transformed, preferably by electroporation, into bacteria. The percentage of positive clones having the 5' tag oligonucleotide is typically estimated to be between 90 and 98% from dot blot analysis.

Following transformation, the libraries are ordered in microtiter plates and sequenced. The *Arabidopsis* library was deposited at the American Type Culture Collection on January 7, 2000 as "*E-coli* liba 010600" under the accession number **PTA-1161**.

EXAMPLE 2: SOUTHERN HYBRIDIZATIONS

The SDFs of the invention can be used in Southern hybridizations as described above. The following describes extraction of DNA from nuclei of plant cells, digestion of the nuclear DNA and separation by length, transfer of the separated fragments to membranes, preparation of probes for hybridization, hybridization and detection of the hybridized probe.

The procedures described herein can be used to isolate related polynucleotides or for diagnostic purposes. Moderate stringency hybridization conditions, as defined above, are described in the present example. These conditions result in detection of hybridization between sequences having at least 70% sequence identity. As described above, the hybridization and wash conditions can be changed to reflect the desired percenatge of sequence identity between probe and target sequences that can be detected.

In the following procedure, a probe for hybridization is produced from two PCR reactions using two primers from genomic sequence of *Arabidopsis thaliana.* As described above, the particular template for generating the probe can be any desired template.

The first PCR product is assessed to validate the size of the primer to assure it is of the expected size. Then the product of the first PCR is used as a template, with the same pair of primers used in the first PCR, in a second PCR that produces a labeled product used as the probe.

Fragments detected by hybridization, or other bands of interest, can be isolated from gels used to separate genomic DNA fragments by known methods for further purification and/or characterization.

Buffers for nuclear DNA extraction

1. 10X HB

	1000 ml	
40 mM spermidine	10.2 g	Spermine (Sigma S-2876) and spermidine (Sigma S-2501)
10 mM spermine	3.5 g	Stabilize chromatin and the nuclear membrane
0.1 M EDTA (disodium)	37.2 g	EDTA inhibits nuclease
0.1 M Tris	12.1 g	Buffer
0.8 M KCl	59.6 g	Adjusts ionic strength for stability of nuclei

Adjust pH to 9.5 with 10 N NaOH. It appears that there is a nuclease present in leaves. Use of pH 9.5 appears to inactivate this nuclease.

2. 2 M sucrose (684 g per 1000 ml)

Heat about half the final volume of water to about 50°C. Add the sucrose slowly then bring the mixture to close to final volume; stir constantly until it has dissolved. Bring the solution to volume.

3. Sarkosyl solution (lyses nuclear membranes)

1000 ml

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N-lauroyl sarcosine (Sarkosyl)	20.0 g
0.1 M Tris	12.1 g
0.04 M EDTA (Disodium)	14.9 g

Adjust the pH to 9.5 after all the components are dissolved and bring up to the proper volume.

4. 20% Triton X-100

80 ml Triton X-100

320 ml 1xHB (w/o β-ME and PMSF)

Prepare in advance; Triton takes some time to dissolve

A. Procedure

1. Prepare 1X "H" buffer (keep ice-cold during use)

	1000 ml	
10X HB	100 ml	
2 M sucrose	250 ml	a non-ionic osmoticum
Water	634 ml	

Added just before use:

100 mM PMSF*	10 ml a protease inhibitor; protects nuclear membrane proteins
ß-mercaptoethanol	1 ml inactivates nuclease by reducing disulfide bonds

*100 mM PMSF

(phenyl methyl sulfonyl fluoride, Sigma P-7626)

(add 0.0875 g to 5 ml 100% ethanol)

2. Homogenize the tissue in a blender (use 300-400 ml of 1xHB per blender). Be sure that you use 5-10 ml of HB buffer per gram of tissue. Blenders generate heat so be

sure to keep the homogenate cold. It is necessary to put the blenders in ice periodically.

3. Add the 20% Triton X-100 (25 ml per liter of homogenate) and gently stir on ice for 20 min. This lyses plastid, but not nuclear, membranes.

4. Filter the tissue suspension through several nylon filters into an ice-cold beaker. The first filtration is through a 250-micron membrane; the second is through an 85-micron membrane; the third is through a 50-micron membrane; and the fourth is through a 20-micron membrane. Use a large funnel to hold the filters. Filtration can be sped up by gently squeezing the liquid through the filters.

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5. Centrifuge the filtrate at 1200 x g for 20 min. at 4°C to pellet the nuclei.

6. Discard the dark green supernatant. The pellet will have several layers to it. One is starch; it is white and gritty. The nuclei are gray and soft. In the early steps, there may be a dark green and somewhat viscous layer of chloroplasts.

Wash the pellets in about 25 ml cold H buffer (with Triton X-100) and resuspend by swirling gently and pipetting. After the pellets are resuspended.

Pellet the nuclei again at 1200 - 1300 x g. Discard the supernatant.

Repeat the wash 3-4 times until the supernatant has changed from a dark green to a pale green. This usually happens after 3 or 4 resuspensions. At this point, the pellet is typically grayish white and very slippery. The Triton X-100 in these repeated steps helps to destroy the chloroplasts and mitochondria that contaminate the prep.

Resuspend the nuclei for a final time in a total of 15 ml of H buffer and transfer the suspension to a sterile 125 ml Erlenmeyer flask.

7. Add 15 ml, dropwise, cold 2% Sarkosyl, 0.1 M Tris, 0.04 M EDTA solution (pH 9.5) while swirling gently. This lyses the nuclei. The solution will become very viscous.

8. Add 30 grams of CsCl and gently swirl at room temperature until the CsCl is in solution. The mixture will be gray, white and viscous.

9. Centrifuge the solution at 11,400 x g at 4ºC for at least 30 min. The longer this spin is, the firmer the protein pellicle.

10. The result is typically a clear green supernatant over a white pellet, and (perhaps) under a protein pellicle. Carefully remove the solution under the protein pellicle and above the pellet. Determine the density of the solution by weighing 1 ml of solution and add CsCl if necessary to bring to 1.57 g/ml. The solution contains dissolved solids (sucrose etc) and the refractive index alone will not be an accurate guide to CsCl concentration.

11. Add 20 µl of 10 mg/ml EtBr per ml of solution.

12. Centrifuge at 184,000 x g for 16 to 20 hours in a fixed-angle rotor.

13. Remove the dark red supernatant that is at the top of the tube with a plastic transfer pipette and discard. Carefully remove the DNA band with another transfer pipette. The DNA band is usually visible in room light; otherwise, use a long wave UV light to locate the band.

14. Extract the ethidium bromide with isopropanol saturated with water and salt. Once the solution is clear, extract at least two more times to ensure that all of the EtBr is gone. Be very gentle, as it is very easy to shear the DNA at this step. This extraction may take a while because the DNA solution tends to be very viscous. If the solution is too viscous, dilute it with TE.

15. Dialyze the DNA for at least two days against several changes (at least three times) of TE (10 mM Tris, 1mM EDTA, pH 8) to remove the cesium chloride.

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16. Remove the dialyzed DNA from the tubing. If the dialyzed DNA solution contains a lot of debris, centrifuge the DNA solution at least at 2500 x g for 10 min. and carefully transfer the clear supernatant to a new tube. Read the A260 concentration of the DNA.

17. Assess the quality of the DNA by agarose gel electrophoresis (1% agarose gel) of the DNA. Load 50 ng and 100 ng (based on the OD reading) and compare it with known and good quality DNA. Undigested lambda DNA and a lambda-HindIII-digested DNA are good molecular weight makers.

Protocol for Digestion of Genomic DNA

<u>Protocol</u>:

1. The relative amounts of DNA for different crop plants that provide approximately a balanced number of genome equivalent is given in Table 3. Note that due to the size of the wheat genome, wheat DNA will be underrepresented. Lambda DNA provides a useful control for complete digestion.

2. Precipitate the DNA by adding 3 volumes of 100% ethanol. Incubate at –20°C for at least two hours. Yeast DNA can be purchased and made up at the necessary concentration, therefore no precipitation is necessary for yeast DNA.

3. Centrifuge the solution at 11,400 x g for 20 min. Decant the ethanol carefully (be careful not to disturb the pellet). Be sure that the residual ethanol is completely removed either by vacuum desiccation or by carefully wiping the sides of the tubes with a clean tissue.

4. Resuspend the pellet in an appropriate volume of water. Be sure the pellet is fully resuspended before proceeding to the next step. This may take about 30 min.

5. Add the appropriate volume of 10X reaction buffer provided by the manufacturer of the restriction enzyme to the resuspended DNA followed by the appropriate volume of enzymes. Be sure to mix it properly by slowly swirling the tubes.

6. Set-up the lambda digestion-control for each DNA that you are digesting.

7. Incubate both the experimental and lambda digests overnight at 37°C. Spin down condensation in a microfuge before proceeding.

8. After digestion, add 2 μl of loading dye (typically 0.25% bromophenol blue, 0.25% xylene cyanol in 15% Ficoll or 30% glycerol) to the lambda-control digests and load in 1% TPE-agarose gel (TPE is 90 mM Tris-phosphate, 2 mM EDTA, pH 8). If the lambda DNA in the lambda control digests are completely digested, proceed with the precipitation of the genomic DNA in the digests.

9. Precipitate the digested DNA by adding 3 volumes of 100% ethanol and incubating in –20°C for at least 2 hours (preferably overnight).

   EXCEPTION: *Arabidopsis* and yeast DNA are digested in an appropriate volume; they don't have to be precipitated.

10. Resuspend the DNA in an appropriate volume of TE (e.g., 22 μl x 50 blots = 1100 μl) and an appropriate volume of 10X loading dye (e.g., 2.4 μl x 50 blots = 120 μl). Be careful in pipetting the loading dye - it is viscous. Be sure you are pipetting the correct volume.

Table 3

Some guide points in digesting genomic DNA.

Species	**Genome Size**	**Size Relative to Arabidopsis**	**Genome Equivalent to 2 μg Arabidopsis DNA**	**Amount of DNA per blot**
Arabidopsis	120 Mb	1X	1X	2 μg
Brassica	1,100 Mb	9.2X	0.54X	10 μg
Corn	2,800 Mb	23.3X	0.43X	20 μg

Cotton	2,300 Mb	19.2X	0.52X	20 μg
Oat	11,300 Mb	94X	0.11X	20 μg
Rice	400 Mb	3.3X	0.75X	5 μg
Soybean	1,100 Mb	9.2X	0.54X	10 μg
Sugarbeet	758 Mb	6.3X	0.8X	10 μg
Sweetclover	1,100 Mb	9.2X	0.54X	10 μg
Wheat	16,000 Mb	133X	0.08X	20 μg
Yeast	15 Mb	0.12X	1X	0.25 μg

Protocol for Southern Blot Analysis

The digested DNA samples are electrophoresed in 1% agarose gels in 1x TPE buffer. Low voltage; overnight separations are preferred. The gels are stained with EtBr and photographed.

1. For blotting the gels, first incubate the gel in 0.25 N HCl (with gentle shaking) for about 15 min.

2. Then briefly rinse with water. The DNA is denatured by 2 incubations. Incubate (with shaking) in 0.5 M NaOH in 1.5 M NaCl for 15 min.

3. The gel is then briefly rinsed in water and neutralized by incubating twice (with shaking) in 1.5 M Tris pH 7.5 in 1.5 M NaCl for 15 min.

4. A nylon membrane is prepared by soaking it in water for at least 5 min, then in 6X SSC for at least 15 min. before use. (20x SSC is 175.3 g NaCl, 88.2 g sodium citrate per liter, adjusted to pH 7.0.)

5. The nylon membrane is placed on top of the gel and all bubbles in between are removed. The DNA is blotted from the gel to the membrane using an absorbent medium, such as paper toweling and 6x SCC buffer. After the transfer, the membrane may be lightly brushed with a gloved hand to remove any agarose sticking to the surface.

6. The DNA is then fixed to the membrane by UV crosslinking and baking at 80°C. The membrane is stored at 4°C until use.

B. Protocol for PCR Amplification of Genomic Fragments in Arabidopsis

Amplification procedures:

1. Mix the following in a 0.20 ml PCR tube or 96-well PCR plate:

Volume	**Stock**	**Final Amount or Conc.**
0.5 µl	~ 10 ng/µl genomic DNA[1]	5 ng
2.5 µl	**10X PCR buffer**	20 mM Tris, 50 mM KCl
0.75 µl	50 mM $MgCl_2$	1.5 mM
1 µl	10 pmol/µl Primer 1 (Forward)	10 pmol
1 µl	10 pmol/µl Primer 2 (Reverse)	10 pmol
0.5 µl	5 mM dNTPs	0.1 mM
0.1 µl	5 units/µl Platinum Taq™ (Life Technologies, Gaithersburg, MD) DNA Polymerase	1 units
(to 25 µl)	**Water**	

2. The template DNA is amplified using a Perkin Elmer 9700 PCR machine:

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[1] Arabidopsis DNA is used in the present experiment, but the procedure is a general one.

1) 94°C for 10 min. followed by

2) 5 cycles:	3) 5 cycles:	4) 25 cycles:
94 °C - 30 sec 62 °C - 30 sec 72 °C - 3 min	94 °C - 30 sec 58 °C - 30 sec 72 °C - 3 min	94 °C - 30 sec 53 °C - 30 sec 72 °C - 3 min

5) 72°C for 7 min. Then the reactions are stopped by chilling to 4°C.

The procedure can be adapted to a multi-well format if necessary.

Quantification and Dilution of PCR Products:

1. The product of the PCR is analyzed by electrophoresis in a 1% agarose gel. A linearized plasmid DNA can be used as a quantification standard (usually at 50, 100, 200, and 400 ng). These will be used as references to approximate the amount of PCR products. HindIII-digested Lambda DNA is useful as a molecular weight marker. The gel can be run fairly quickly; e.g., at 100 volts. The standard gel is examined to determine that the size of the PCR products is consistent with the expected size and if there are significant extra bands or smeary products in the PCR reactions.

2. The amounts of PCR products can be estimated on the basis of the plasmid standard.

3. For the small number of reactions that produce extraneous bands, a small amount of DNA from bands with the correct size can be isolated by dipping a sterile 10-μl tip into the band while viewing though a UV Transilluminator. The small amount of agarose gel (with the DNA fragment) is used in the labeling reaction.

C. Protocol for PCR-DIG-Labeling of DNA

Solutions:

Reagents in PCR reactions (diluted PCR products, 10X PCR Buffer, 50 mM $MgCl_2$, 5 U/μl Platinum Taq Polymerase, and the primers)

10X dNTP + DIG-11-dUTP **[1:5]**: (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.65 mM dTTP, 0.35 mM DIG-11-dUTP)

10X dNTP + DIG-11-dUTP **[1:10]**: (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.81 mM dTTP, 0.19 mM DIG-11-dUTP)

10X dNTP + DIG-11-dUTP **[1:15]**: (2 mM dATP, 2 mM dCTP, 2 mM dGTP, 1.875 mM dTTP, 0.125 mM DIG-11-dUTP)

TE buffer (10 mM Tris, 1 mM EDTA, pH 8)

Maleate buffer: In 700 ml of deionized distilled water, dissolve 11.61 g maleic acid and 8.77 g NaCl. Add NaOH to adjust the pH to 7.5. Bring the volume to 1 L. Stir for 15 min. and sterilize.

10% blocking solution: In 80 ml deionized distilled water, dissolve 1.16g maleic acid. Next, add NaOH to adjust the pH to 7.5. Add 10 g of the blocking reagent powder (Boehringer Mannheim, Indianapolis, IN, Cat. no. 1096176). Heat to 60°C while stirring to dissolve the powder. Adjust the volume to 100 ml with water. Stir and sterilize.

1% blocking solution: Dilute the 10% stock to 1% using the maleate buffer.

Buffer 3 (100 mM Tris, 100 mM NaCl, 50 mM $MgCl_2$, pH9.5). Prepared from autoclaved solutions of 1M Tris pH 9.5, 5 M NaCl, and 1 M $MgCl_2$ in autoclaved distilled water.

Procedure:

1. PCR reactions are performed in 25 μl volumes containing:

PCR buffer	1X
$MgCl_2$	1.5 mM
10X dNTP + DIG-11-dUTP	1X (please see the note below)
Platinum Taq™ Polymerase	1 unit
10 pg probe DNA	
10 pmol primer 1	

Note:	Use for:
10X dNTP + DIG-11-dUTP (1:5)	< 1 kb
10X dNTP + DIG-11-dUTP (1:10)	1 kb to 1.8 kb
10X dNTP + DIG-11-dUTP (1:15)	> 1.8 kb

2. The PCR reaction uses the following amplification cycles:

1) 94°C for 10 min.

2) 5 cycles:	3) 5 cycles:	4) 25 cycles:
95°C - 30 sec 61°C - 1 min 73°C - 5 min	95°C - 30 sec 59°C - 1 min 75°C - 5 min	95°C - 30 sec 51°C - 1 min 73°C - 5 min

5) 72°C for 8 min. The reactions are terminated by chilling to 4°C (hold).

3. The products are analyzed by electrophoresis- in a 1% agarose gel, comparing to an aliquot of the unlabelled probe starting material.

4. The amount of DIG-labeled probe is determined as follows:

Make serial dilutions of the diluted control DNA in dilution buffer (TE: 10 mM Tris and 1 mM EDTA, pH 8) as shown in the following table:

DIG-labeled control DNA starting conc.	**Stepwise Dilution**	**Final Conc. (Dilution Name)**
5 ng/μl	1 μl in 49 μl TE	100 pg/μl (A)
100 pg/μl (A)	25 μl in 25 μl TE	50 pg/μl (B)
50 pg/μl (B)	25 μl in 25 μl TE	25 pg/μl (C)
25 pg/μl (C)	20 μl in 30 μl TE	10 pg/μl (D)

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a. Serial deletions of a DIG-labeled standard DNA ranging from 100 pg to 10 pg are spotted onto a positively charged nylon membrane, marking the membrane lightly with a pencil to identify each dilution.

b. Serial dilutions (e.g., 1:50, 1:2500, 1:10,000) of the newly labeled DNA probe are spotted.

c. The membrane is fixed by UV crosslinking.

d. The membrane is wetted with a small amount of maleate buffer and then incubated in 1% blocking solution for 15 min at room temp.

e. The labeled DNA is then detected using alkaline phosphatase conjugated anti-DIG antibody (Boehringer Mannheim, Indianapolis, IN, cat. no. 1093274) and an NBT substrate according to the manufacture's instruction.

f. Spot intensities of the control and experimental dilutions are then compared to estimate the concentration of the PCR-DIG-labeled probe.

D. Prehybridization and Hybridization of Southern Blots

Solutions:

100% Formamide purchased from Gibco

20X SSC ($1X = 0.15$ M NaCl, 0.015 M Na_3citrate)

per L: 175 g NaCl

87.5 g Na_3citrate·$2H_20$

20% Sarkosyl (N-lauroyl-sarcosine)

20% SDS (sodium dodecyl sulphate)

10% Blocking Reagent: In 80 ml deionized distilled water, dissolve 1.16 g maleic acid. Next, add NaOH to adjust the pH to 7.5. Add 10 g of the blocking reagent powder. Heat to 60°C while stirring to dissolve the powder. Adjust the volume to 100 ml with water. Stir and sterilize.

Prehybridization Mix:

Final Concentration	**Components**	**Volume (per 100 ml)**	**Stock**
50%	Formamide	50 ml	100%
5X	SSC	25 ml	20X
0.1%	Sarkosyl	0.5 ml	20%
0.02%	SDS	0.1 ml	20%
2%	Blocking Reagent	20 ml	10%
	Water	4.4 ml	

General Procedures:

1. Place the blot in a heat-sealable plastic bag and add an appropriate volume of prehybridization solution (30 ml/$100cm^2$) at room temperature. Seal the bag with a heat sealer, avoiding bubbles as much as possible. Lay down the bags in a large plastic tray (one tray can accommodate at least 4–5 bags). Ensure that the bags are

lying flat in the tray so that the prehybridization solution is evenly distributed throughout the bag. Incubate the blot for at least 2 hours with gentle agitation using a waver shaker.

2. Denature DIG-labeled DNA probe by incubating for 10 min. at 98°C using the PCR machine and immediately cool it to 4°C.

3. Add probe to prehybridization solution (25 ng/ml; 30 ml = 750 ng total probe) and mix well but avoid foaming. Bubbles may lead to background.

4. Pour off the prehybridization solution from the hybridization bags and add new prehybridization and probe solution mixture to the bags containing the membrane.

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5. Incubate with gentle agitation for at least 16 hours.

6. Proceed to medium stringency post-hybridization wash:

   Three times for 20 min. each with gentle agitation using 1X SSC, 1% SDS at 60°C.

   All wash solutions must be prewarmed to 60°C. Use about 100 ml of wash solution per membrane.

   To avoid background keep the membranes fully submerged to avoid drying in spots; agitate sufficiently to avoid having membranes stick to one another.

7. After the wash, proceed to immunological detection and CSPD development.

E. Procedure for Immunological Detection with CSPD

<u>Solutions</u>:

Buffer 1:	Maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl; adjusted to pH 7.5 with NaoH)

Washing buffer:	Maleic acid buffer with 0.3% (v/v) Tween 20.
Blocking stock solution	10% blocking reagent in buffer 1. Dissolve (10X concentration): blocking reagent powder (Boehringer Mannheim, Indianapolis, IN, cat. no. 1096176) by constantly stirring on a 65°C heating block or heat in a microwave, autoclave and store at 4°C.
Buffer 2 (1X blocking solution):	Dilute the stock solution 1:10 in Buffer 1.
Detection buffer:	0.1 M Tris, 0.1 M NaCl, pH 9.5

<u>Procedure</u>:

1. After the post-hybridization wash the blots are briefly rinsed (1-5 min.) in the maleate washing buffer with gentle shaking.

2. Then the membranes are incubated for 30 min. in Buffer 2 with gentle shaking.

3. Anti-DIG-AP conjugate (Boehringer Mannheim, Indianapolis, IN, cat. no. 1093274) at 75 mU/ml (1:10,000) in Buffer 2 is used for detection. 75 ml of solution can be used for 3 blots.

4. The membrane is incubated for 30 min. in the antibody solution with gentle shaking.

5. The membrane are washed twice in washing buffer with gentle shaking. About 250 mls is used per wash for 3 blots.
6. The blots are equilibrated for 2–5 min in 60 ml detection buffer.

7. Dilute CSPD (1:200) in detection buffer. (This can be prepared ahead of time and stored in the dark at 4°C).

The following steps must be done individually. Bags (one for detection and one for exposure) are generally cut and ready before doing the following steps.

8. The blot is carefully removed from the detection buffer and excess liquid removed without drying the membrane. The blot is immediately placed in a bag and 1.5 ml of CSPD solution is added. The CSPD solution can be spread over the membrane. Bubbles present at the edge and on the surface of the blot are typically removed by gentle rubbing. The membrane is incubated for 5 min. in CSPD solution.

9. Excess liquid is removed and the membrane is blotted briefly (DNA side up) on Whatman 3MM paper. Do not let the membrane dry completely.

10. Seal the damp membrane in a hybridization bag and incubate for 10 min at 37°C to enhance the luminescent reaction.

11. Expose for 2 hours at room temperature to X-ray film. Multiple exposures can be taken. Luminescence continues for at least 24 hours and signal intensity increases during the first hours.

<u>Example 3: Transformation of Carrot Cells</u>

Transformation of plant cells can be accomplished by a number of methods, as described above. Similarly, a number of plant genera can be regenerated from tissue culture following transformation. Transformation and regeneration of carrot cells as described herein is illustrative.

Single cell suspension cultures of carrot (*Daucus carota*) cells are established from hypocotyls of cultivar Early Nantes in B_5 growth medium (O.L. Gamborg et al., *Plant Physiol.* 45:372 (1970)) plus 2,4-D and 15 mM $CaCl_2$ (B_5 -44 medium) by methods known in the art. The suspension cultures are subcultured by adding 10 ml of the suspension culture to 40 ml of B_5-44 medium in 250 ml flasks every 7 days and are maintained in a shaker at 150 rpm at 27 °C in the dark.

The suspension culture cells are transformed with exogenous DNA as described by Z. Chen et al. *Plant Mol. Bio.* 36:163 (1998). Briefly, 4-days post-subculture cells are incubated with cell wall digestion solution containing 0.4 M sorbitol, 2% driselase, 5mM MES (2-[N-

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Morpholino] ethanesulfonic acid) pH 5.0 for 5 hours. The digested cells are pelleted gently at 60 xg for 5 min. and washed twice in W5 solution containing 154 mM NaCl, 5 mM KCl, 125 mM $CaCl_2$ and 5mM glucose, pH 6.0. The protoplasts are suspended in MC solution containing 5 mM MES, 20 mM $CaCl_2$, 0.5 M mannitol, pH 5.7 and the protoplast density is adjusted to about 4 x 10^6 protoplasts per ml.

15-60 μg of plasmid DNA is mixed with 0.9 ml of protoplasts. The resulting suspension is mixed with 40% polyethylene glycol (MW 8000, PEG 8000), by gentle inversion a few times at room temperature for 5 to 25 min. Protoplast culture medium known in the art is added into the PEG-DNA-protoplast mixture. Protoplasts are incubated in the culture medium for 24 hour to 5 days and cell extracts can be used for assay of transient expression of the introduced gene. Alternatively, transformed cells can be used to produce transgenic callus, which in turn can be used to produce transgenic plants, by methods known in the art. See, for example, Nomura and Komamine, *Plt. Phys.* 79:988-991 (1985), *Identification and Isolation of Single Cells that Produce Somatic Embryos in Carrot Suspension Cultures.*

An additional deposit, PTA-1411, of an *E. coli* Library, *E. coli*LibA021800, was made at the American Type Culture Collection in Manassas, Virginia, USA on February 22, 2000 to meet the requirements of Budapest Treaty for the international recognition of the deposit of microorganisms. This deposit was assigned ATCC accession no. PTA-1411.

The invention being thus described, it will be apparent to one of ordinary skill in the art that various modifications of the materials and methods for practicing the invention can be made. Such modifications are to be considered within the scope of the invention as defined by the following claims.

Each of the references from the patent and periodical literature cited herein is hereby expressly incorporated in its entirety by such citation.

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TABLE 1

```
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    Intr   24949    25090          +         0
    Intr   25275    25355          +         0
    Intr   25618    25746          +         0
    Intr   25852    25929          +         0
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     Term   11112   11469         +        0

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>1532162       /32937

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     Intr   23189   23323         +        0
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        Intr    23415    23494          +          0
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    >1532162        /15968

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>1707006         /26506

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      Intr    22887    22993         +         0
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>1707006         /40748

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    Intr   68592   68875        +         0
    Intr   68968   69064        +         0
Intr   69314   69419         +       0
Intr   69514   69596         +       0
Intr   69689   69834         +       0
Intr   69966   70071         +       0
Intr   70216   70275         +       0
Term   70361   70496         +       0

>1707006       /101081

len =     976   nex =        1

Sngl   75101   74126         -       0

>1785673       /23693

len =     622   nex =        1

Sngl   31275   31896         +       0

>1871173       /38610

len =    2054   nex =        8

Init   12146   12237         +       0
Intr   12284   12483         +       0
Intr   12573   12704         +       0
Intr   12790   12866         +       0
Intr   12970   13105         +       0
Intr   13186   13326         +       0
Intr   13424   13482         +       0
Term   13584   13650         +       0

>1871173       /10969

len =    1038   nex =        2

Term   49483   49183         -       0
Init   50220   49576         -       0

>1871173       /16493

len =    3870   nex =        7

Init   50368   50540         +       0
Intr   50630   50680         +       0
Intr   51247   51298         +       0
Intr   51370   51428         +       0
Intr   51546   51591         +       0
Intr   52100   52233         +       0
Term   54023   54234         +       0

>1877523       /96448

len =     822   nex =        4

Init  105150  105228         +       0
Intr  105315  105428         +       0
Intr  105500  105535         +       0
Term  105630  105953         +       0
```

```
>1877523        /2677

   len =     670   nex =       1

    Sngl   21255   20592        -       0

>1877523        /1693

   len =     710   nex =       2

    Term   21139   20646        -       0
    Init   21355   21247        -       0

>1877523        /40042

   len =    2369   nex =       8

    Term   38060   37972        -       0
    Intr   38794   38656        -       0
    Intr   38997   38927        -       0
    Intr   39148   39104        -       0
    Intr   39328   39239        -       0
    Intr   39526   39438        -       0
    Intr   39689   39614        -       0
    Init   40034   39798        -       0

>1877523        /35733

   len =    1245   nex =       3

    Term   53914   53583        -       0
    Intr   54335   54159        -       0
    Init   54827   54574        -       0

>1877523        /34291

   len =     809   nex =       1

    Sngl   61137   61281        +       0

>1877523        /2979

   len =     766   nex =       2

    Init   61146   61281        +       0
    Term   61565   61911        +       0

>1931636        /93598

   len =    1589   nex =       3

    Init  111855  112746        +       0
    Intr  112845  112949        +       0
    Term  113156  113443        +       0

>1931636        /40765

   len =    1821   nex =       3

    Term   50015   49475        -       0
```

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```
  Intr   50253   50130         -        0
  Init   51295   50557         -        0

>1931636       /20637

 len =     644   nex =       1

  Sngl   63596   62953         -        0

>1931636       /14648

 len =     503   nex =       1

  Sngl   97733   97231         -        0

>1946354       /1391

 len =    4584   nex =      11

  Term   12119   11739         -        0
  Intr   12281   12213         -        0
  Intr   12535   12455         -        0
  Intr   12756   12682         -        0
  Intr   13005   12873         -        0
  Intr   13304   13257         -        0
  Intr   13613   13401         -        0
  Intr   13994   13833         -        0
  Intr   14593   14363         -        0
  Intr   15009   14680         -        0
  Init   15456   15157         -        0

>1946354       /7619

 len =     939   nex =       2

  Init   31875   32384         +        0
  Term   32537   32813         +        0

>1946354       /34999

 len =    1078   nex =       2

  Init   33182   33416         +        0
  Term   33743   34259         +        0

>1946354       /39560

 len =     674   nex =       1

  Sngl   41592   42265         +        0

>1946354       /41046

 len =     730   nex =       1

  Sngl   57609   58323         +        0

>1946354       /1820

 len =    1190   nex =       2
```

```
    Term    7729    6909          -        0
    Init    8098    7816          -        0

>1946354        /22671

   len =    1583   nex =        2

    Init   83167   83385          +        0
    Term   83523   83614          +        0

>2062153        /38051

   len =    1491   nex =        3

    Term   15272   14834          -        0
    Intr   15841   15386          -        0
    Init   16324   16026          -        0

>2062153        /119458

   len =    1513   nex =        1

    Sngl   16220   16026          -        0

>2062153        /157474

   len =    1497   nex =        3

    Term   15272   14858          -        0
    Intr   15841   15386          -        0
    Init   16220   16026          -        0

>2062153        /30056

   len =    1520   nex =        3

    Term   15272   14836          -        0
    Intr   15841   15386          -        0
    Init   16220   16026          -        0

>2062153        /42777

   len =    1450   nex =        2

    Term   24390   23947          -        0
    Init   25283   24512          -        0

>2062153        /6448

   len =    1481   nex =        3

    Term   24390   23947          -        0
    Intr   24955   24512          -        0
    Init   25427   25053          -        0

>2062153        /12715

   len =    1976   nex =        3
   Term   55961   55118        -       0
   Intr   56262   56051        -       0
   Init   57093   56580        -       0

>2062153        /30003

  len =     2057   nex =       3

   Init    7382    7843        +       0
   Intr    7929    8378        +       0
   Term    8469    8866        +       0

>2062153        /32293

  len =      790   nex =       1

   Sngl   69530   68750        -       0

>2062153        /29750

  len =     2112   nex =       3

   Init   76786   77284        +       0
   Intr   77663   77774        +       0
   Term   77921   78394        +       0

>2088638        /9398

  len =      616   nex =       1

   Sngl  103573  102958        -       0

>2088638        /6732

  len =     1632   nex =       2

   Term   17390   16530        -       0
   Init   18161   17822        -       0

>2088638        /39048

  len =     2533   nex =       7

   Init   24452   24782        +       0
   Intr   25154   25378        +       0
   Intr   25457   25551        +       0
   Intr   25633   25822        +       0
   Intr   25917   26041        +       0
   Intr   26186   26401        +       0
   Term   26486   26984        +       0

>2088638        /33701

  len =     1515   nex =       3

   Term   32027   31685        -       0
   Intr   32312   32109        -       0
   Init   32802   32388        -       0

>2088638        /15207
```

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```
   len =     2110    nex =        4

    Term   50426   50181         -         0
    Intr   50656   50514         -         0
    Intr   51540   51487         -         0
    Init   52290   52051         -         0

>2088638       /5504

   len =     2820    nex =       10

    Term   52859   52504         -         0
    Intr   53066   52943         -         0
    Intr   53260   53159         -         0
    Intr   53424   53356         -         0
    Intr   53674   53567         -         0
    Intr   53905   53851         -         0
    Intr   54431   54301         -         0
    Intr   54618   54544         -         0
    Intr   54880   54803         -         0
    Init   55058   54973         -         0

>2088638       /35056

   len =     1510    nex =        1

    Sngl   70686   69178         -         0

>2088638       /32440

   len =      919    nex =        3

    Init   80756   80853         +         0
    Intr   81026   81170         +         0
    Term   81258   81674         +         0

>2088638       /5046

   len =     1647    nex =        3

    Init   95145   95511         +         0
    Intr   95860   96013         +         0
    Term   96327   96791         +         0

>2098816       /31252

   len =      704    nex =        1

    Sngl   35121   35824         +         0

>2098816       /15292

   len =     1279    nex =        1

    Sngl   39507   40333         +         0

>2098816       /36730

   len =     2135    nex =        9
```

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```
     Init   43827   44181        +       0
     Intr   44267   44314        +       0
     Intr   44406   44582        +       0
     Intr   44668   44818        +       0
     Intr   44908   44994        +       0
     Intr   45079   45203        +       0
     Intr   45282   45400        +       0
     Intr   45483   45594        +       0
     Term   45685   45961        +       0

>2098816       /8716

    len =    1090   nex =       5

     Init   44941   44994        +       0
     Intr   45079   45203        +       0
     Intr   45282   45400        +       0
     Intr   45483   45594        +       0
     Term   45685   46007        +       0

>2098816       /36216

    len =    2338   nex =       6

     Init   58990   59535        +       0
     Intr   59663   59944        +       0
     Intr   60031   60178        +       0
     Intr   60282   60367        +       0
     Intr   60894   60971        +       0
     Term   61070   61327        +       0

>2098816       /42713

    len =    2280   nex =       6

     Init   59046   59535        +       0
     Intr   59663   59944        +       0
     Intr   60031   60178        +       0
     Intr   60282   60367        +       0
     Intr   60894   60971        +       0
     Term   61070   61325        +       0

>2098816       /36286

    len =     643   nex =       1

     Sngl    6052    5410        -       0

>2098816       /38820

    len =     756   nex =       1

     Sngl    6188    5433        -       0

>2098816       /38170

    len =    2445   nex =       6

     Term   63428   62916        -       0
```

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```
          Intr    63750    63522          -         0
          Intr    63933    63894          -         0
          Intr    64507    64381          -         0
          Intr    64935    64803          -         0
          Init    65360    65060          -         0

     >2098816         /40254

        len =      628    nex =         1

          Sngl    69008    68744          -         0

     >2098816         /17126

        len =      811    nex =         2

          Term    69008    68666          -         0
          Init    69476    69328          -         0

     >2098816         /122497

        len =      359    nex =         1

          Sngl    70110    69752          -         0

     >2098816         /36543

        len =     1173    nex =         1

          Sngl    77771    76602          -         0

     >2098816         /17357

        len =     2350    nex =         6

          Init    88159    88663          +         0
          Intr    88942    89027          +         0
          Intr    89118    89341          +         0
          Intr    89580    89646          +         0
          Intr    90016    90126          +         0
          Term    90323    90506          +         0

     >2098816         /31770

        len =     1183    nex =         3

          Term    90853    90569          -         0
          Intr    91066    90933          -         0
          Init    91751    91546          -         0

     >2104523         /21952

        len =     2710    nex =         2

          Term    71964    70632          -         0
          Init    73339    72021          -         0

     >2104523         /34676

        len =     3970    nex =        12
```

```
      Term   76257   75990         -        0
      Intr   76489   76350         -        0
      Intr   77518   77169         -        0
      Intr   77737   77610         -        0
      Intr   77956   77828         -        0
      Intr   78109   78031         -        0
      Intr   78360   78197         -        0
      Intr   78636   78451         -        0
      Intr   78894   78763         -        0
      Intr   79089   78998         -        0
      Intr   79279   79180         -        0
      Init   79954   79652         -        0

>2160132        /9002

     len =     1994   nex =       4

      Term   38308   37073         -        0
      Intr   38529   38400         -        0
      Intr   38751   38614         -        0
      Init   39066   38884         -        0

>2160132        /18804

     len =     1584   nex =       1

      Sngl   60820   59237         -        0

>2160132        /21783

     len =      415   nex =       1

      Sngl   78298   78712         +        0

>2160132        /21416

     len =      698   nex =       1

      Sngl   79001   78304         -        0

>2160132        /15957

     len =     2326   nex =      10

      Term   88656   88489         -        0
      Intr   88915   88769         -        0
      Intr   89076   89020         -        0
      Intr   89255   89214         -        0
      Intr   89496   89416         -        0
      Intr   89765   89687         -        0
      Intr   89957   89886         -        0
      Intr   90142   90042         -        0
      Intr   90343   90314         -        0
      Init   90814   90417         -        0

>2160132        /41375

     len =     2353   nex =      10
```

```
   Term   88656   88487        -         0
   Intr   88915   88769        -         0
   Intr   89076   89020        -         0
   Intr   89255   89214        -         0
   Intr   89496   89416        -         0
   Intr   89765   89687        -         0
   Intr   89957   89886        -         0
   Intr   90142   90042        -         0
   Intr   90343   90314        -         0
   Init   90839   90417        -         0

>2160155       /17761

  len =     3312   nex =       7

   Term   15073   14667        -         0
   Intr   15288   15145        -         0
   Intr   15853   15737        -         0
   Intr   16067   15930        -         0
   Intr   16314   16190        -         0
   Intr   17071   16986        -         0
   Init   17685   17547        -         0

>2160155       /6295

  len =     1644   nex =       2

   Init   44387   45479        +         0
   Term   45550   46030        +         0

>2160155       /22067

  len =     2477   nex =       7

   Term   52597   52279        -         0
   Intr   52839   52684        -         0
   Intr   53020   52925        -         0
   Intr   53302   53110        -         0
   Intr   54097   53469        -         0
   Intr   54467   54189        -         0
   Init   54755   54540        -         0

>2160155       /6319

  len =     3299   nex =      11

   Init   60441   60770        +         0
   Intr   61340   61399        +         0
   Intr   61506   61619        +         0
   Intr   61883   61948        +         0
   Intr   62027   62134        +         0
   Intr   62237   62320        +         0
   Intr   62639   62740        +         0
   Intr   62828   62941        +         0
   Intr   63016   63096        +         0
   Intr   63191   63310        +         0
   Term   63481   63739        +         0

>2160155       /17081
```

```
    len =     1390    nex =        2

      Term     5724     5111          -          0
      Init     6498     6293          -          0

>2160155        /39525

    len =     2602    nex =        6

      Init     7274     7423          +          0
      Intr     7512     7572          +          0
      Intr     7673     7725          +          0
      Intr     7845     7946          +          0
      Intr     8057     8546          +          0
      Term     8659     9410          +          0

>2160155        /6642

    len =      823    nex =        2

      Init    76276    76526          +          0
      Term    76743    77098          +          0

>2160155        /8575

    len =      851    nex =        2

      Init    76277    76526          +          0
      Term    76743    77127          +          0

>2160155        /34772

    len =     1361    nex =        3

      Init     7845     7946          +          0
      Intr     8057     8546          +          0
      Term     8659     9205          +          0

>2160155        /23319

    len =      798    nex =        2

      Term    86139    86067          -          0
      Init    86864    86267          -          0

>2182285        /21725

    len =     2410    nex =        4

      Init    10500    10780          +          0
      Intr    11596    11657          +          0
      Intr    12371    12411          +          0
      Term    12536    12907          +          0

>2182285        /2118

    len =      508    nex =        2

      Init    33841    33970          +          0
      Term    34088    34262          +          0
```

```
>2182285        /25136

   len =      674   nex =        2

    Init   36937   37065         +         0
    Term   37294   37610         +         0

>2182285        /108302

   len =      610   nex =        2

    Init   38504   38637         +         0
    Term   38787   39106         +         0

>2182285        /1264

   len =      819   nex =        1

    Sngl   40950   40138         -         0

>2182285        /27763

   len =     2303   nex =        5

    Term   51059   50550         -         0
    Intr   51488   51406         -         0
    Intr   51733   51567         -         0
    Intr   52004   51818         -         0
    Init   52852   52510         -         0

>2182285        /13186

   len =     2050   nex =        0

>2182285        /27609

   len =      957   nex =        4

    Term   97439   97181         -         0
    Intr   97605   97537         -         0
    Intr   97796   97693         -         0
    Init   98125   97940         -         0

>2182286        /37761

   len =     1581   nex =        2

    Term   12027   10760         -         0
    Init   12340   12113         -         0

>2182286        /34835

   len =     2290   nex =        6

    Term   20929   20543         -         0
    Intr   21150   21014         -         0
    Intr   21441   21240         -         0
    Intr   21635   21537         -         0
    Intr   22248   22162         -         0
```

```
     Init   22824   22577         -        0

 >2182286        /15161

    len =     336   nex =       1

     Sngl   32955   32620         -        0

 >2182286        /3538

    len =    1090   nex =       5

     Term   56957   56571         -        0
     Intr   57090   57034         -        0
     Intr   57291   57192         -        0
     Intr   57436   57378         -        0
     Init   57657   57525         -        0

 >2182286        /31705

    len =    1400   nex =       1

     Sngl   61850   60451         -        0

 >2182287        /13008

    len =    2683   nex =      13

     Init   15455   15581         +        0
     Intr   15687   15748         +        0
     Intr   15834   15911         +        0
     Intr   15991   16066         +        0
     Intr   16164   16234         +        0
     Intr   16347   16448         +        0
     Intr   16539   16628         +        0
     Intr   16756   16830         +        0
     Intr   17067   17099         +        0
     Intr   17201   17272         +        0
     Intr   17463   17525         +        0
     Intr   17658   17773         +        0
     Term   17832   18137         +        0

 >2182287        /14016

    len =    1166   nex =       1

     Sngl   33340   33469         +        0

 >2182287        /35042

    len =     497   nex =       1

     Sngl   33706   33927         +        0

 >2182287        /20858

    len =    1183   nex =       1

     Sngl   50135   49403         -        0
```

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```
>2182287         /2985

    len =     1215    nex =        2

     Init    66989    67367          +         0
     Term    67700    68203          +         0

>2182287         /20754

    len =     1074    nex =        2

     Init    67134    67367          +         0
     Term    67700    68203          +         0

>2182287         /13385

    len =     3085    nex =       14

     Init    70516    70728          +         0
     Intr    71073    71225          +         0
     Intr    71312    71410          +         0
     Intr    71569    71670          +         0
     Intr    71750    71893          +         0
     Intr    72009    72103          +         0
     Intr    72210    72339          +         0
     Intr    72415    72519          +         0
     Intr    72624    72730          +         0
     Intr    72806    72857          +         0
     Intr    72941    73030          +         0
     Intr    73120    73204          +         0
     Intr    73290    73354          +         0
     Term    73441    73600          +         0

>2182287         /121535

    len =      153    nex =        1

     Sngl    88279    88127          -         0

>2182287         /103034

    len =      910    nex =        3

     Term    97140    96927          -         0
     Intr    97606    97230          -         0
     Init    97824    97721          -         0

>2182289         /27197

    len =     2831    nex =       11

     Term    14016    13844          -         0
     Intr    14182    14117          -         0
     Intr    14330    14282          -         0
     Intr    14688    14621          -         0
     Intr    14820    14775          -         0
     Intr    15017    14924          -         0
     Intr    15178    15116          -         0
     Intr    15465    15343          -         0
     Intr    15654    15621          -         0
    Intr   16127   16011        -        0
    Init   16674   16500        -        0

>2182289        /205500

   len =    1030   nex =       1

    Sngl   52945   51919        -        0

>2182289        /31372

   len =    1810   nex =       7

    Term   58360   58263        -        0
    Intr   58757   58601        -        0
    Intr   59020   58889        -        0
    Intr   59232   59120        -        0
    Intr   59487   59328        -        0
    Intr   59636   59613        -        0
    Init   60069   59921        -        0

>2182289        /38858

   len =    1690   nex =       3

    Term   85015   84307        -        0
    Intr   85533   85226        -        0
    Init   85988   85678        -        0

>2191126        /28640

   len =    1810   nex =       3

    Init  105687  105961        +        0
    Intr  106179  106465        +        0
    Term  106570  107490        +        0

>2191126        /1204

   len =    1690   nex =       1

    Sngl  110532  112213        +        0

>2191126        /41187

   len =    2551   nex =       7

    Term  115853  115628        -        0
    Intr  116345  116037        -        0
    Intr  116498  116418        -        0
    Intr  116825  116751        -        0
    Intr  116990  116904        -        0
    Intr  117192  117090        -        0
    Init  118178  117485        -        0

>2191126        /21195

   len =    2566   nex =       7

    Term  115853  115637        -        0
```

```
        Intr  116345  116037          -         0
        Intr  116498  116418          -         0
        Intr  116825  116751          -         0
        Intr  116990  116904          -         0
        Intr  117192  117090          -         0
        Init  118202  117485          -         0

   >2191126        /19141

        len =     3438   nex =       14

        Term   25095   24742          -         0
        Intr   25245   25180          -         0
        Intr   25409   25338          -         0
        Intr   25625   25512          -         0
        Intr   25812   25720          -         0
        Intr   25961   25899          -         0
        Intr   26152   26042          -         0
        Intr   26360   26247          -         0
        Intr   26604   26506          -         0
        Intr   26756   26691          -         0
        Intr   26948   26853          -         0
        Intr   27119   27035          -         0
        Intr   27350   27203          -         0
        Init   28179   28046          -         0

   >2191126        /22919

        len =     1497   nex =       4

        Init   28448   28746          +         0
        Intr   29035   29235          +         0
        Intr   29321   29463          +         0
        Term   29617   29944          +         0

   >2191126        /117191

        len =      253   nex =       1

        Sngl   66070   66322          +         0

   >2191126        /7653

        len =     1991   nex =       6

        Term    5264    4896          -         0
        Intr    5520    5337          -         0
        Intr    5933    5601          -         0
        Intr    6260    6123          -         0
        Intr    6458    6329          -         0
        Init    6886    6590          -         0

   >2191126        /41270

        len =     1008   nex =       4

        Term   79378   79212          -         0
        Intr   79532   79461          -         0
        Intr   79730   79623          -         0
        Init   79919   79819          -         0
```

```
>2191126       /94836

   len =    1131   nex =       5

    Term   79378   79190          -         0
    Intr   79532   79461          -         0
    Intr   79730   79623          -         0
    Intr   79919   79819          -         0
    Init   80320   80142          -         0

>2191126       /12604

   len =    1177   nex =       5

    Term   79378   79212          -         0
    Intr   79532   79461          -         0
    Intr   79730   79623          -         0
    Intr   79919   79819          -         0
    Init   80388   80142          -         0

>2191126       /16533

   len =    2830   nex =      12

    Init   90009   90076          +         0
    Intr   90175   90222          +         0
    Intr   90315   90410          +         0
    Intr   90483   90563          +         0
    Intr   90659   90700          +         0
    Intr   90784   90947          +         0
    Intr   91030   91078          +         0
    Intr   91166   91214          +         0
    Intr   91306   91445          +         0
    Intr   91538   91615          +         0
    Intr   91709   91834          +         0
    Term   91909   92323          +         0

>2191157       /5457

   len =     688   nex =       2

    Term  110545  110202          -         0
    Init  110889  110723          -         0

>2191157       /39714

   len =     520   nex =       1

    Sngl   24526   25045          +         0

>2191157       /37336

   len =    1558   nex =       2

    Init   26629   26769          +         0
    Term   27064   27170          +         0

>2191157       /17739
```

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```
len =    2326   nex =      11

 Term    1098     904        -        0
 Intr    1303    1201        -        0
 Intr    1501    1418        -        0
 Intr    1698    1603        -        0
 Intr    1848    1798        -        0
 Intr    2076    1936        -        0
 Intr    2220    2164        -        0
 Intr    2391    2317        -        0
 Intr    2739    2467        -        0
 Intr    2894    2835        -        0
 Init    3094    3002        -        0

>2191157        /21258

len =    2364   nex =       9

 Init   35554   35767        +        0
 Intr   35854   35917        +        0
 Intr   36017   36231        +        0
 Intr   36362   36538        +        0
 Intr   36622   36696        +        0
 Intr   36794   36895        +        0
 Intr   37265   37376        +        0
 Intr   37474   37620        +        0
 Term   37753   37793        +        0

>2191157        /42174

len =     540   nex =       1

 Sngl   59287   59826        +        0

>2191157        /27625

len =     732   nex =       2

 Init   80900   81166        +        0
 Term   81274   81631        +        0

>2191157        /41361

len =    2136   nex =       2

 Init   83526   83731        +        0
 Term   83861   84187        +        0

>2191157        /32265

len =    2008   nex =       2

 Init   83526   83731        +        0
 Term   83861   84181        +        0

>2191157        /2495

len =    2795   nex =       5

 Init   92543   92875        +        0
```

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```
    Intr   93634   93776         +       0
    Intr   94054   94077         +       0
    Intr   94512   94714         +       0
    Term   94965   95337         +       0

>2191181        /38304

   len =     2070   nex =        4

    Init    1742    2050         +       0
    Intr    2468    2686         +       0
    Intr    2758    2844         +       0
    Term    3193    3219         +       0

>2191181        /23239

   len =      988   nex =        3

    Term    4337    3802         -       0
    Intr    4497    4418         -       0
    Init    4789    4601         -       0

>2191181        /30935

   len =     1455   nex =        0

>2213606        /6503

   len =     1974   nex =        4

    Init   15815   16171         +       0
    Intr   16373   16842         +       0
    Intr   16925   17188         +       0
    Term   17281   17788         +       0

>2213606        /10990

   len =      413   nex =        1

    Sngl   18252   17840         -       0

>2213606        /38093

   len =      490   nex =        1

    Sngl   27032   27514         +       0

>2213606        /23231

   len =      700   nex =        1

    Sngl   45292   44593         -       0

>2213606        /31944

   len =      559   nex =        1

    Sngl   49930   49372         -       0

>2244747        /16846
```

```
   len =     2017    nex =        2

    Init   12786    13565         +        0
    Term   13854    14802         +        0

>2244747        /38987

   len =      134    nex =        1

    Sngl   14762    14895         +        0

>2244747        /17977

   len =      610    nex =        1

    Sngl   16599    15997         -        0

>2244747        /19172

   len =      610    nex =        1

    Sngl   16614    16009         -        0

>2244747        /30129

   len =      813    nex =        1

    Sngl  176792   177114         +        0

>2244747        /195

   len =      805    nex =        1

    Sngl  176309   177113         +        0

>2244747        /101734

   len =      340    nex =        1

    Sngl  198899   199238         +        0

>2244747        /126389

   len =     1776    nex =        8

    Init   48741    48903         +        0
    Intr   48995    49057         +        0
    Intr   49141    49207         +        0
    Intr   49296    49396         +        0
    Intr   49486    49530         +        0
    Intr   49614    49895         +        0
    Intr   49983    50085         +        0
    Term   50189    50516         +        0

>2244747        /25991

   len =     1850    nex =        8

    Init   48741    48903         +        0
    Intr   48995   49057        +        0
    Intr   49141   49207        +        0
    Intr   49296   49396        +        0
    Intr   49486   49530        +        0
    Intr   49614   49895        +        0
    Intr   49983   50085        +        0
    Term   50189   50590        +        0

>2244747       /99093

   len =     430   nex =       3

    Init   48743   48903        +        0
    Intr   48995   49057        +        0
    Term   49141   49172        +        0

>2244747       /7346

   len =     550   nex =       1

    Sngl   51305   50761        -        0

>2244747       /13520

   len =     522   nex =       1

    Sngl   53660   53139        -        0

>2244747       /18697

   len =     817   nex =       2

    Term   56326   55871        -        0
    Init   56687   56413        -        0

>2244747       /35186

   len =    1525   nex =       5

    Term   56326   55870        -        0
    Intr   56685   56413        -        0
    Intr   56884   56777        -        0
    Intr   57220   56989        -        0
    Init   57394   57303        -        0

>2244747       /39975

   len =    2277   nex =       7

    Term   56326   55859        -        0
    Intr   56685   56413        -        0
    Intr   56884   56777        -        0
    Intr   57220   56989        -        0
    Intr   57530   57303        -        0
    Intr   57816   57621        -        0
    Init   58135   57936        -        0

>2244747       /108308

   len =    2306   nex =       6
```

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```
    Term   58896   58494        -        0
    Intr   59256   58984        -        0
    Intr   59446   59412        -        0
    Intr   59994   59535        -        0
    Intr   60270   60075        -        0
    Init   60799   60608        -        0

>2244747        /34967

   len =     1692   nex =        3

    Init   78644   78978        +        0
    Intr   79811   79967        +        0
    Term   80055   80335        +        0

>2244747        /29662

   len =     2324   nex =        4

    Term    6181    5707        -        0
    Intr    6376    6275        -        0
    Intr    6858    6468        -        0
    Init    8030    7268        -        0
>2244747        /10852

   len =      948   nex =        3

    Term   95484   95087        -        0
    Intr   95756   95563        -        0
    Init   96034   95845        -        0

>2244747        /33554

   len =     1225   nex =        3

    Term   95484   94981        -        0
    Intr   95756   95563        -        0
    Init   96205   95845        -        0

>2244788        /33860

   len =      894   nex =        2

    Init  119066  119340        +        0
    Term  119433  119959        +        0

>2244788        /4232

   len =     1570   nex =        3

    Term   11837   11610        -        0
    Intr   12997   12874        -        0
    Init   13171   13086        -        0

>2244788        /20129

   len =     1736   nex =        4
```

```
     Init  134496  134633         +        0
     Intr  134785  134908         +        0
     Intr  135250  135306         +        0
     Term  135918  136231         +        0

>2244788        /4905

    len =    1532   nex =       4

     Init  134547  134633         +        0
     Intr  134785  134908         +        0
     Intr  135250  135306         +        0
     Term  135918  136078         +        0

>2244788        /18255

    len =    1917   nex =       4

     Term   11837   11553         -        0
     Intr   12997   12874         -        0
     Intr   13171   13086         -        0
     Init   13469   13401         -        0

>2244788        /42223

    len =    1270   nex =       2

     Init  141770  141970         +        0
     Term  142713  143034         +        0

>2244788        /21908

    len =     865   nex =       2

     Term  172609  172540         -        0
     Init  173404  172806         -        0

>2244788        /95834

    len =     932   nex =       4

     Init  176283  176507         +        0
     Intr  176602  176703         +        0
     Intr  176785  176939         +        0
     Term  176951  177214         +        0

>2244788        /31495

    len =    1150   nex =       5

     Init  177820  177887         +        0
     Intr  178110  178208         +        0
     Intr  178295  178347         +        0
     Intr  178445  178518         +        0
     Term  178797  178969         +        0

>2244788        /40073

    len =    1761   nex =       5
```

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```
    Term  182960  182681          -         0
    Intr  183144  183074          -         0
    Intr  183352  183228          -         0
    Intr  183544  183430          -         0
    Init  184441  183731          -         0

>2244788        /2738

   len =     1855    nex =        7

    Term  182960  182701          -         0
    Intr  183144  183074          -         0
    Intr  183352  183228          -         0
    Intr  183544  183430          -         0
    Intr  183825  183731          -         0
    Intr  184012  183901          -         0
    Init  184555  184343          -         0

>2244788        /18153

   len =     1337    nex =        4

    Term     744     549          -         0
    Intr     903     829          -         0
    Intr    1232    1053          -         0
    Init    1885    1804          -         0

>2244788        /16319

   len =     1732    nex =        7

    Term  188526  188214          -         0
    Intr  188710  188640          -         0
    Intr  188914  188790          -         0
    Intr  189112  188998          -         0
    Intr  189340  189246          -         0
    Intr  189532  189421          -         0
    Init  189945  189850          -         0

>2244788        /34477

   len =      790    nex =        4

    Term   26188   26035          -         0
    Intr   26496   26276          -         0
    Intr   26702   26590          -         0
    Init   26822   26779          -         0

>2244788        /37809

   len =     2215    nex =       10

    Term   29960   29503          -         0
    Intr   30139   30054          -         0
    Intr   30309   30235          -         0
    Intr   30490   30388          -         0
    Intr   30687   30606          -         0
    Intr   30881   30790          -         0
    Intr   31057   30969          -         0
    Intr   31236   31156          -         0
    Intr   31450   31336         -        0
    Init   31717   31579         -        0

>2244788       /9870

  len =    1700   nex =       6

    Term   45280   45046         -        0
    Intr   45431   45380         -        0
    Intr   45545   45518         -        0
    Intr   46149   46080         -        0
    Intr   46413   46313         -        0
    Init   46745   46519         -        0

>2244788       /40736

  len =    1713   nex =       5

    Init   57948   58133         +        0
    Intr   58560   58765         +        0
    Intr   58850   58930         +        0
    Intr   59012   59174         +        0
    Term   59262   59660         +        0

>2244788       /1718

  len =    1844   nex =       5

    Term   60276   59985         -        0
    Intr   60467   60369         -        0
    Intr   60644   60555         -        0
    Intr   60856   60742         -        0
    Init   61828   61672         -        0

>2244788       /94503

  len =    1930   nex =       5

    Term   60276   59949         -        0
    Intr   60467   60369         -        0
    Intr   60644   60555         -        0
    Intr   60856   60742         -        0
    Init   61875   61672         -        0

>2244788       /28978

  len =     921   nex =       1

    Sngl   63706   62786         -        0

>2244788       /36844

  len =    1309   nex =       1

    Sngl   78815   80123         +        0

>2244788       /42933

  len =    2960   nex =       6
```

```
      Init   92232   92765        +      0
      Intr   92959   93121        +      0
      Intr   93567   93743        +      0
      Intr   93831   93914        +      0
      Intr   94438   94519        +      0
      Term   94602   95191        +      0

>2244829       /38042

    len =    2717   nex =       9

      Init  103735  104049        +      0
      Intr  104329  104423        +      0
      Intr  104545  104609        +      0
      Intr  104833  104876        +      0
      Intr  105212  105295        +      0
      Intr  105486  105639        +      0
      Intr  105738  105920        +      0
      Intr  106013  106069        +      0
      Term  106159  106451        +      0

>2244829       /293

    len =     315   nex =       1

      Sngl  114012  113698        -      0

>2244829       /40074

    len =    1498   nex =       2

      Term  115095  113973        -      0
      Init  115470  115294        -      0

>2244829       /38411

    len =    1796   nex =       2

      Term  115095  113698        -      0
      Init  115493  115294        -      0

>2244829       /10518

    len =    2190   nex =       8

      Init  116378  116531        +      0
      Intr  116787  116872        +      0
      Intr  116953  117024        +      0
      Intr  117143  117180        +      0
      Intr  117526  117569        +      0
      Intr  117791  117837        +      0
      Intr  117992  118166        +      0
      Term  118269  118567        +      0

>2244829       /29288

    len =     492   nex =       1

      Sngl  131227  130736        -      0
```

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```
>2244829        /24175

   len =      332    nex =        1

    Sngl  136899  137230          +        0

>2244829        /17179

   len =      450    nex =        1

    Sngl  136899  137332          +        0

>2244829        /99523

   len =      346    nex =        1

    Sngl  136900  137245          +        0

>2244829        /37184

   len =      624    nex =        0

>2244829        /126602

   len =      654    nex =        1

    Sngl  136900  137553          +        0

>2244829        /15384

   len =      627    nex =        1

    Sngl  136904  137530          +        0

>2244829        /26797

   len =      628    nex =        1

    Sngl  136904  137531          +        0

>2244829        /36129

   len =      739    nex =        1

    Sngl  199828  200566          +        0

>2244829        /24266

   len =     1908    nex =        3

    Init    65354    65621        +        0
    Intr    65713    65836        +        0
    Term    66807    67261        +        0

>2244829        /31856

   len =      897    nex =        3

    Init    70117    70500        +        0
    Intr    70585    70611        +        0
```

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```
                                         899
     Term   70696   71013        +         0

   >2244829        /30327

     len =      711    nex =       1

     Sngl   82258   82968        +         0

   >2244829        /33166

     len =      650    nex =       1

     Sngl   82303   82952        +         0

   >2244829        /42848

     len =     2473    nex =       9

     Term   83367   83062        -         0
     Intr   83556   83476        -         0
     Intr   83703   83644        -         0
     Intr   83890   83811        -         0
     Intr   84071   84020        -         0
     Intr   84306   84169        -         0
     Intr   84661   84398        -         0
     Intr   84799   84742        -         0
     Init   84996   84887        -         0

   >2244829        /22861

     len =      611    nex =       1

     Sngl   85902   86512        +         0

   >2244829        /25333

     len =     2115    nex =       3

     Term   87340   86629        -         0
     Intr   87618   87443        -         0
     Init   88743   87767        -         0

   >2244829        /117350

     len =     1760    nex =       8

     Term   93545   93422        -         0
     Intr   93819   93710        -         0
     Intr   93998   93936        -         0
     Intr   94168   94094        -         0
     Intr   94368   94276        -         0
     Intr   94573   94469        -         0
     Intr   94861   94740        -         0
     Init   95181   94950        -         0

   >2244870        /2163

     len =     1517    nex =       1

     Sngl   13507   15023        +         0
```

```
>2244870        /15641

   len =     1853    nex =        7

    Init     2352     2569          +         0
    Intr     2668     2781          +         0
    Intr     2862     2957          +         0
    Intr     3057     3099          +         0
    Intr     3174     3326          +         0
    Intr     3408     3476          +         0
    Term     3843     4204          +         0

>2244870        /35290

   len =     1090    nex =        2

    Term    33366    33045          -         0
    Init    34113    33943          -         0

>2244870        /18642

   len =      867    nex =        2

    Term     4431     4071          -         0
    Init     4937     4513          -         0

>2244870        /30852

   len =      513    nex =        1

    Sngl    70945    70433          -         0

>2244870        /36205

   len =     1210    nex =        1

    Sngl    71644    70435          -         0

>2244870        /30929

   len =      867    nex =        1

    Sngl    84563    85414          +         0

>2244901        /32219

   len =      644    nex =        1

    Sngl   100297   100940          +         0

>2244901        /101301

   len =     1235    nex =        2

    Init    12251    12597          +         0
    Term    13371    13485          +         0

>2244901        /15334
```

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```
     len =     2089    nex =        4

      Init   12251    12597          +         0
      Intr   13371    13484          +         0
      Intr   13678    13835          +         0
      Term   13944    14339          +         0

>2244901        /14485

     len =     1048    nex =        2

      Term  136645   136202          -         0
      Init  137249   136976          -         0

>2244901        /8916

     len =      761    nex =        2

      Init  146636   146871          +         0
      Term  146912   147396          +         0

>2244901        /22637

     len =     1930    nex =        7

      Init  150934   151112          +         0
      Intr  151807   151845          +         0
      Intr  151938   151991          +         0
      Intr  152091   152144          +         0
      Intr  152269   152322          +         0
      Intr  152417   152488          +         0
      Term  152622   152862          +         0

>2244901        /5455

     len =      550    nex =        1

      Sngl  153514   154059          +         0

>2244901        /25390

     len =     1731    nex =        3

      Term  156239   156216          -         0
      Intr  156385   156332          -         0
      Init  157099   156997          -         0

>2244901        /39757

     len =     1489    nex =        5

      Term  164193   163773          -         0
      Intr  164487   164293          -         0
      Intr  164750   164603          -         0
      Intr  164938   164832          -         0
      Init  165261   165017          -         0

>2244901        /113295

     len =      250    nex =        1
```

```
    Sngl  165261  165021         -        0

>2244901        /43007

  len =     3418    nex =        9

    Init  181307  182180         +        0
    Intr  182482  182558         +        0
    Intr  182639  182732         +        0
    Intr  182817  182915         +        0
    Intr  183212  183301         +        0
    Intr  183400  183519         +        0
    Intr  183767  183870         +        0
    Intr  184163  184235         +        0
    Term  184397  184724         +        0

>2244901        /8381

  len =      928    nex =        2

    Init  197128  197392         +        0
    Term  197699  198055         +        0

>2244901        /35383

  len =     1690    nex =        1

    Sngl   23032   21343         -        0

>2244901        /12451

  len =     2050    nex =        4

    Init   29261   29459         +        0
    Intr   29681   29785         +        0
    Intr   29969   30397         +        0
    Term   30959   31303         +        0

>2244901        /8234

  len =      855    nex =        4

    Term   33518   33296         -        0
    Intr   33802   33633         -        0
    Intr   34017   33880         -        0
    Init   34150   34103         -        0

>2244901        /33073

  len =     3028    nex =        2

    Init    4164    4631         +        0
    Term    6071    7191         +        0

>2244901        /307

  len =     1838    nex =        8

    Init   44565   44888         +        0
```

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```
     Intr    44976    45044          +         0
     Intr    45145    45198          +         0
     Intr    45288    45327          +         0
     Intr    45414    45512          +         0
     Intr    45595    45819          +         0
     Intr    45902    46023          +         0
     Term    46120    46402          +         0

>2244901        /19122

    len =     1766    nex =        8

     Init    44638    44888          +         0
     Intr    44976    45044          +         0
     Intr    45145    45198          +         0
     Intr    45288    45327          +         0
     Intr    45414    45512          +         0
     Intr    45595    45819          +         0
     Intr    45902    46023          +         0
     Term    46120    46403          +         0

>2244901        /37345

    len =     1379    nex =        3

     Init    55027    55308          +         0
     Intr    55387    55671          +         0
     Term    55759    56179          +         0

>2244901        /26019

    len =     1750    nex =        3

     Init    77747    78039          +         0
     Intr    78780    78906          +         0
     Term    79065    79492          +         0

>2244901        /933

    len =     1415    nex =        2

     Init    86075    86413          +         0
     Term    86998    87489          +         0

>2244950        /12629

    len =     3346    nex =       10

     Term   100982   100625          -         0
     Intr   101466   101106          -         0
     Intr   101718   101591          -         0
     Intr   102002   101874          -         0
     Intr   102439   102360          -         0
     Intr   102690   102527          -         0
     Intr   102958   102773          -         0
     Intr   103205   103074          -         0
     Intr   103432   103291          -         0
     Init   103970   103568          -         0

>2244950        /40414
```

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```
    len =     2150    nex =        6

     Term  109338  109067        -        0
     Intr  109551  109489        -        0
     Intr  109708  109646        -        0
     Intr  109850  109803        -        0
     Intr  110001  109939        -        0
     Init  111043  110961        -        0

>2244950         /30227

    len =     2050    nex =        6

     Term  109338  109187        -        0
     Intr  109551  109489        -        0
     Intr  109708  109646        -        0
     Intr  109850  109803        -        0
     Intr  110001  109939        -        0
     Init  111043  110961        -        0

>2244950         /5714

    len =     1403    nex =        7

     Init  124186  124326        +        0
     Intr  124418  124469        +        0
     Intr  124596  124670        +        0
     Intr  124766  124794        +        0
     Intr  124968  125001        +        0
     Intr  125082  125152        +        0
     Term  125251  125588        +        0

>2244950         /33513

    len =     1593    nex =        4

     Init  138127  138644        +        0
     Intr  138739  138858        +        0
     Intr  138934  139180        +        0
     Term  139256  139719        +        0

>2244950         /19028

    len =      638    nex =        2

     Init  139024  139180        +        0
     Term  139256  139661        +        0

>2244950         /21894

    len =     1030    nex =        1

     Sngl  146832  145803        -        0

>2244950         /7605

    len =      814    nex =        2

     Term  167332  166714        -        0
```

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```
    Init  167527  167451         -        0

>2244950        /3176

   len =     1423    nex =        2

    Term  167332  166764         -        0
    Init  167934  167451         -        0

>2244950        /41791

   len =     1479    nex =        3

    Term  167332  166712         -        0
    Intr  167934  167451         -        0
    Init  168190  168116         -        0

>2244950        /12256

   len =     1716    nex =        4

    Term  169269  169015         -        0
    Intr  169606  169448         -        0
    Intr  170335  170260         -        0
    Init  170730  170607         -        0

>2244950        /6723

   len =     1536    nex =        4

    Init  171676  171958         +        0
    Intr  172224  172415         +        0
    Intr  172496  172661         +        0
    Term  172740  173211         +        0

>2244950        /124835

   len =      978    nex =        1

    Sngl   18831   19808         +        0

>2244950        /40793

   len =     1247    nex =        3

    Term  193189  192906         -        0
    Intr  193587  193266         -        0
    Init  194152  193673         -        0

>2244950        /2803

   len =     1824    nex =        3

    Init    2896    3184         +        0
    Intr    3571    3676         +        0
    Term    4403    4719         +        0

>2244950        /9209

   len =      573    nex =        1
```

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```
    Sngl   31137   30565         -        0

>2244950        /29655

  len =      682   nex =        1

    Sngl   34486   35167         +        0

>2244950        /40913

  len =     2079   nex =        7

    Init     4949    5128        +        0
    Intr     5254    5419        +        0
    Intr     5498    5550        +        0
    Intr     5911    5973        +        0
    Intr     6366    6416        +        0
    Intr     6516    6630        +        0
    Term     6687    7027        +        0

>2244950        /18234

  len =     1950   nex =        6

    Init   61059   61335         +        0
    Intr   61420   61550         +        0
    Intr   61714   61791         +        0
    Intr   61882   61926         +        0
    Intr   62016   62060         +        0
    Term   62293   62389         +        0

>2244950        /32203

  len =     1510   nex =        6

    Init     7376    7454        +        0
    Intr     7542    7577        +        0
    Intr     7707    7844        +        0
    Intr     7939    8012        +        0
    Intr     8418    8486        +        0
    Term     8556    8884        +        0

>2244950        /31782

  len =     1211   nex =        1

    Sngl   84183   82973         -        0

>2244950        /17019

  len =     2897   nex =        2

    Term   84672   82981         -        0
    Init   85877   85235         -        0

>2244950        /109560

  len =      397   nex =        1
```

```
    Sngl    95604   96000         +        0

>2244991        /7101

   len =     1300   nex =        5

    Term    99473   99160         -        0
    Intr    99674   99597         -        0
    Intr    99851   99788         -        0
    Intr   100015   99939         -        0
    Init   100216  100170         -        0

>2244991        /14136

   len =     1251   nex =        1

    Sngl   133001  131751         -        0

>2244991        /24611

   len =     1275   nex =        6

    Init   144816  144916         +        0
    Intr   144996  145065         +        0
    Intr   145153  145209         +        0
    Intr   145299  145360         +        0
    Intr   145408  145507         +        0
    Term   145593  145964         +        0

>2244991        /5546

   len =     1163   nex =        3

    Term   157187  156808         -        0
    Intr   157430  157305         -        0
    Init   157970  157545         -        0

>2244991        /8212

   len =     1254   nex =        0

>2244991        /40778

   len =      879   nex =        3

    Init   163368  163492         +        0
    Intr   163658  163757         +        0
    Term   163863  164240         +        0

>2244991        /23771

   len =     1377   nex =        4

    Term   164902  164507         -        0
    Intr   165186  164989         -        0
    Intr   165666  165500         -        0
    Init   165883  165813         -        0

>2244991        /16525
```

```
    len =      810    nex =        2

     Init  172277  172503          +        0
     Term  172604  173086          +        0

>2244991        /22084

    len =     1450    nex =        2

     Init  177203  177333          +        0
     Term  177407  177827          +        0

>2244991        /157870

    len =      342    nex =        1

     Sngl   17882   17541          -        0

>2244991        /5686

    len =     2453    nex =       10

     Term  194540  194396          -        0
     Intr  194759  194680          -        0
     Intr  194888  194843          -        0
     Intr  195027  194971          -        0
     Intr  195163  195105          -        0
     Intr  195344  195244          -        0
     Intr  195623  195502          -        0
     Intr  195980  195929          -        0
     Intr  196138  196058          -        0
     Init  196848  196213          -        0

>2244991        /2505

    len =      623    nex =        1

     Sngl   27093   26471          -        0

>2244991        /7632

    len =     1210    nex =        3

     Term   36794   36385          -        0
     Intr   37205   37073          -        0
     Init   37590   37308          -        0

>2244991        /30471

    len =     1883    nex =        6

     Term   39363   38946          -        0
     Intr   39486   39437          -        0
     Intr   39651   39570          -        0
     Intr   39806   39736          -        0
     Intr   40168   40098          -        0
     Init   40371   40292          -        0

>2244991        /17535
```

```
    len =      585   nex =       1

     Sngl   43288   43872         +        0

>2244991        /17553

    len =      628   nex =       2

     Init   44575   44786         +        0
     Term   44876   45202         +        0

>2244991        /16090

    len =      634   nex =       2

     Init   44583   44786         +        0
     Term   44876   45216         +        0

>2244991        /31946

    len =      562   nex =       1

     Sngl   66524   65963         -        0

>2244991        /6580

    len =      509   nex =       1

     Sngl   70265   69757         -        0

>2244991        /17851

    len =     1752   nex =       5

     Term   71484   71210         -        0
     Intr   71754   71636         -        0
     Intr   71898   71846         -        0
     Intr   72484   72429         -        0
     Init   72626   72579         -        0

>2244991        /92054

    len =      587   nex =       1

     Sngl    8564    9150         +        0

>2245031        /92144

    len =      444   nex =       1

     Sngl  125198  125641         +        0

>2245031        /30087

    len =      822   nex =       1

     Sngl  125198  126019         +        0

>2245031        /118011
```

```
      len =      355    nex =        1

        Sngl  125287  125641          +        0

   >2245031        /91870

      len =     1970    nex =        4

        Init  144106  144256          +        0
        Intr  144641  144768          +        0
        Intr  145143  145253          +        0
        Term  145583  146075          +        0

   >2245031        /36017

      len =     3647    nex =        8

        Term  154141  153926          -        0
        Intr  155021  154948          -        0
        Intr  155252  155139          -        0
        Intr  155661  155584          -        0
        Intr  155955  155829          -        0
        Intr  156204  156149          -        0
        Intr  156561  156358          -        0
        Init  157572  157241          -        0

   >2245031        /7834

      len =     3010    nex =       12

        Init  157780  157908          +        0
        Intr  157993  158125          +        0
        Intr  158517  158604          +        0
        Intr  158708  158784          +        0
        Intr  159068  159107          +        0
        Intr  159412  159497          +        0
        Intr  159590  159671          +        0
        Intr  159798  159854          +        0
        Intr  159938  159976          +        0
        Intr  160067  160137          +        0
        Intr  160354  160407          +        0
        Term  160554  160780          +        0

   >2245031        /114540

      len =     3018    nex =       11

        Init  157780  157908          +        0
        Intr  157993  158125          +        0
        Intr  158517  158604          +        0
        Intr  158708  158784          +        0
        Intr  159068  159497          +        0
        Intr  159590  159671          +        0
        Intr  159798  159854          +        0
        Intr  159938  159976          +        0
        Intr  160067  160137          +        0
        Intr  160354  160407          +        0
        Term  160554  160797          +        0

   >2245031        /110681
```

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```
     len =      466   nex =        2

      Init  172709  172801          +        0
      Term  172906  173174          +        0

>2245031        /142850

     len =      610   nex =        1

      Sngl  173847  173242          -        0

>2245031        /42533

     len =     1533   nex =        4

      Init   17415   17660          +        0
      Intr   17764   18062          +        0
      Intr   18331   18410          +        0
      Term   18499   18947          +        0

>2245031        /36882

     len =     2299   nex =        5

      Term  173963  173241          -        0
      Intr  174262  174007          -        0
      Intr  174516  174406          -        0
      Intr  174824  174614          -        0
      Init  175539  174923          -        0

>2245031        /14613

     len =      673   nex =        1

      Sngl   20501   19829          -        0

>2245031        /831

     len =      850   nex =        3

      Init   39954   40111          +        0
      Intr   40198   40248          +        0
      Term   40330   40796          +        0

>2245031        /14223

     len =      638   nex =        1

      Sngl   43095   43370          +        0

>2245031        /35772

     len =     1663   nex =        1

      Sngl   48986   49948          +        0

>2245073        /158661

     len =      739   nex =        1
```

```
    Sngl  102245  101507         -        0

>2245073       /34167

   len =    1019   nex =       3

    Init  104868  105196         +        0
    Intr  105282  105361         +        0
    Term  105463  105866         +        0

>2245073       /36603

   len =    4481   nex =      11

    Term    6893    6584         -        0
    Intr    7287    7083         -        0
    Intr    7700    7618         -        0
    Intr    8129    7990         -        0
    Intr    8424    8266         -        0
    Intr    9480    8479         -        0
    Intr    9839    9542         -        0
    Intr   10132    9928         -        0
    Intr   10433   10351         -        0
    Intr   10748   10609         -        0
    Init   11064   10945         -        0

>2245073       /37223

   len =    4483   nex =      11

    Term    6893    6584         -        0
    Intr    7287    7083         -        0
    Intr    7700    7618         -        0
    Intr    8129    7990         -        0
    Intr    8424    8266         -        0
    Intr    9480    8479         -        0
    Intr    9839    9542         -        0
    Intr   10132    9928         -        0
    Intr   10433   10351         -        0
    Intr   10748   10609         -        0
    Init   11066   10945         -        0

>2245073       /6042

   len =     959   nex =       1

    Sngl  124096  125054         +        0

>2245073       /35156

   len =    2133   nex =       7

    Init  136139  136418         +        0
    Intr  136654  136948         +        0
    Intr  137036  137101         +        0
    Intr  137200  137329         +        0
    Intr  137421  137579         +        0
    Intr  137703  137753         +        0
    Term  137855  138271         +        0
```

```
>2245073       /154342

   len =     111   nex =       1

     Sngl  140364  140254         -        0

>2245073       /3258

   len =    2050   nex =       8

     Term  138586  138326         -        0
     Intr  138787  138684         -        0
     Intr  139039  138884         -        0
     Intr  139188  139117         -        0
     Intr  139338  139291         -        0
     Intr  139469  139422         -        0
     Intr  139680  139608         -        0
     Init  140370  140183         -        0

>2245073       /2161

   len =    1690   nex =       5

     Init  145051  145144         +        0
     Intr  145227  145544         +        0
     Intr  145712  145798         +        0
     Intr  145888  146021         +        0
     Term  146416  146733         +        0

>2245073       /17120

   len =     464   nex =       2

     Init  145081  145144         +        0
     Term  145227  145544         +        0

>2245073       /29150

   len =    1072   nex =       3

     Init  168520  168924         +        0
     Intr  169023  169160         +        0
     Term  169230  169591         +        0

>2245073       /23025

   len =    2715   nex =       8

     Init  181224  181382         +        0
     Intr  181935  181992         +        0
     Intr  182407  182489         +        0
     Intr  182789  183061         +        0
     Intr  183152  183204         +        0
     Intr  183325  183405         +        0
     Intr  183502  183614         +        0
     Term  183704  183938         +        0

>2245073       /19505
```

```
   len =     2035    nex =        4

    Init  189969  190426         +        0
    Intr  190764  190988         +        0
    Intr  191116  191225         +        0
    Term  191315  191480         +        0

>2245073        /31781

   len =     1939    nex =        4

    Init  190050  190426         +        0
    Intr  190764  190988         +        0
    Intr  191116  191225         +        0
    Term  191315  191480         +        0

>2245073        /36521

   len =      730    nex =        1

    Sngl  190098  190332         +        0

>2245073        /39872

   len =     2135    nex =        4

    Init  192291  192840         +        0
    Intr  193297  193492         +        0
    Intr  193589  193720         +        0
    Term  194093  194425         +        0

>2245073        /6709

   len =     1058    nex =        2

    Term  198909  198442         -        0
    Init  199499  199146         -        0

>2245073        /94923

   len =      739    nex =        2

    Init   20607   20828         +        0
    Term   20918   21345         +        0

>2245073        /24997

   len =      530    nex =        1

    Sngl   26357   25828         -        0

>2245073        /33509

   len =     1450    nex =        2

    Init   38766   39446         +        0
    Term   39638   40214         +        0

>2245073        /35260
```

```
        len =     1557    nex =       3

         Term    43961    43535          -         0
         Intr    44176    44048          -         0
         Init    45091    44398          -         0

    >2245073        /27500

        len =     1700    nex =       1

         Sngl    51675    51471          -         0

    >2245073        /99796

        len =     1150    nex =       2

         Init    64024    64466          +         0
         Term    64647    65171          +         0

    >2245073        /31538

        len =     1278    nex =       4

         Term    79423    79066          -         0
         Intr    79725    79528          -         0
         Intr    80213    80047          -         0
         Init    80343    80313          -         0

    >2245073        /26448

        len =     2146    nex =       8

         Term    87600    87509          -         0
         Intr    87818    87699          -         0
         Intr    88211    88116          -         0
         Intr    88333    88295          -         0
         Intr    88636    88458          -         0
         Intr    88765    88726          -         0
         Intr    88913    88854          -         0
         Init    89406    89167          -         0

    >2245126        /39922

        len =     2134    nex =       5

         Term    28671    27817          -         0
         Intr    28825    28745          -         0
         Intr    28988    28913          -         0
         Intr    29183    29080          -         0
         Init    29950    29830          -         0

    >2245126        /37533

        len =     1873    nex =       7

         Init    30483    30887          +         0
         Intr    30977    31070          +         0
         Intr    31153    31292          +         0
         Intr    31365    31439          +         0
         Intr    31521    31678          +         0
```

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```
    Intr   31762   31823         +        0
    Term   31972   32355         +        0

>2245126        /42815

   len =    1514   nex =       4

    Init   56618   56988         +        0
    Intr   57254   57524         +        0
    Intr   57621   57791         +        0
    Term   57887   58131         +        0

>2252639        /36439

   len =    2305   nex =      12

    Term  112752  112679         -        0
    Intr  112953  112837         -        0
    Intr  113158  113042         -        0
    Intr  113355  113254         -        0
    Intr  113539  113444         -        0
    Intr  113704  113623         -        0
    Intr  113928  113814         -        0
    Intr  114069  114018         -        0
    Intr  114227  114147         -        0
    Intr  114489  114328         -        0
    Intr  114748  114572         -        0
    Init  114983  114885         -        0

>2252639        /32628

   len =    8176   nex =       7

    Term  112752  112549         -        0
    Intr  112953  112837         -        0
    Intr  113158  113042         -        0
    Intr  113355  113254         -        0
    Intr  113539  113444         -        0
    Intr  113704  113623         -        0
    Init  113928  113814         -        0

>2252639        /7870

   len =    2062   nex =       9

    Init   55275   55373         +        0
    Intr   55679   55864         +        0
    Intr   55943   56072         +        0
    Intr   56168   56248         +        0
    Intr   56342   56529         +        0
    Intr   56624   56719         +        0
    Intr   56822   56915         +        0
    Intr   57043   57162         +        0
    Term   57257   57336         +        0

>2252639        /42847

   len =    2459   nex =       3

    Init   64066   64204         +        0
```

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```
          Intr    65296    65804          +        0
          Term    65895    66271          +        0

     >2252639         /20756

         len =       561    nex =        1

          Sngl    66935    66375          -        0

     >2252639         /8355

         len =       619    nex =        1

          Sngl    67016    66406          -        0

     >2252639         /104398

         len =       114    nex =        1

          Sngl    67655    67768          +        0

     >2252639         /34829

         len =      1550    nex =        5

          Term    72152    71686          -        0
          Intr    72324    72213          -        0
          Intr    72574    72402          -        0
          Intr    72867    72664          -        0
          Init    73235    73005          -        0

     >2252639         /34276

         len =      2157    nex =        6

          Term    76139    75823          -        0
          Intr    76346    76218          -        0
          Intr    76530    76444          -        0
          Intr    76771    76626          -        0
          Intr    76952    76898          -        0
          Init    77979    77037          -        0

     >2252639         /11108

         len =       539    nex =        1

          Sngl    79342    78804          -        0

     >2252639         /1269

         len =      1433    nex =        3

          Term    79851    79679          -        0
          Intr    80212    80012          -        0
          Init    80700    80396          -        0

     >2252639         /5476

         len =       835    nex =        3
```

09555526 .051600

```
    Init   85064   85271        +       0
    Intr   85376   85455        +       0
    Term   85554   85898        +       0

>2252639       /35833

   len =     873   nex =       3

    Init   85064   85271        +       0
    Intr   85376   85455        +       0
    Term   85554   85936        +       0

>2252639       /1810

   len =     878   nex =       3

    Init   85064   85271        +       0
    Intr   85376   85455        +       0
    Term   85554   85941        +       0

>2252639       /17857

   len =     910   nex =       3

    Init   85064   85271        +       0
    Intr   85376   85455        +       0
    Term   85554   85972        +       0

>2252639       /10862

   len =     864   nex =       3

    Init   85068   85271        +       0
    Intr   85376   85455        +       0
    Term   85554   85931        +       0

>2252639       /22773

   len =    2008   nex =       2

    Term   92196   90691        -       0
    Init   92698   92411        -       0

>2252823       /11106

   len =    1289   nex =       1

    Sngl  107171  108459        +       0

>2252823       /25765

   len =     315   nex =       1

    Sngl    1671    1357        -       0

>2252823       /38970

   len =    2486   nex =       1

    Sngl   29968   30145        +       0
```

09555326 .061500

```
>2252823        /15741

    len =     3070    nex =        3

      Init   29968    30145          +         0
      Intr   30436    30547          +         0
      Term   30642    31104          +         0

>2252823        /28637

    len =     2900    nex =        3

      Init   35493    36349          +         0
      Intr   36852    37326          +         0
      Term   37673    38392          +         0

>2252823        /21038

    len =      495    nex =        1

      Sngl   37895    38389          +         0

>2252823        /35506

    len =      582    nex =        1

      Sngl   50035    49454          -         0

>2252823        /39479

    len =     1604    nex =        3

      Term   56402    56064          -         0
      Intr   57185    56486          -         0
      Init   57649    57493          -         0

>2252823        /36326

    len =     2392    nex =        3

      Term   64455    64054          -         0
      Intr   64734    64625          -         0
      Init   65205    64824          -         0

>2252823        /31027

    len =     1150    nex =        2

      Init   94085    94153          +         0
      Term   94219    95230          +         0

>2252848        /111719

    len =      733    nex =        1

      Sngl   46064    45332          -         0

>2252848        /11036
```

09595326 .061600

```
      len =      790    nex =        1

       Sngl    46089   45304         -         0

   >2252848         /3204

      len =      833    nex =        1

       Sngl    60597   61429         +         0

   >2252848         /22161

      len =      670    nex =        1

       Sngl    63070   63731         +         0

   >2252848         /22348

      len =      740    nex =        1

       Sngl    65608   64869         -         0

   >2252848         /28082

      len =     1216    nex =        3

        Init   80915    80991        +         0
        Intr   81337    81552        +         0
        Term   81645    81897        +         0

   >2252848         /26442

      len =     1210    nex =        3

        Init   80915    80991        +         0
        Intr   81337    81552        +         0
        Term   81645    81895        +         0

   >2252848         /37305

      len =     1575    nex =        4

        Term   91905    91570        -         0
        Intr   92168    92002        -         0
        Intr   92528    92246        -         0
        Init   92758    92613        -         0

   >2252848         /37175

      len =     2050    nex =        3

        Term   95449    94674        -         0
        Intr   95668    95551        -         0
        Init   96720    96101        -         0

   >2262097         /22611

      len =     1439    nex =        4

        Init      31      168        +         0
```

09525516 .061600

```
        Intr      253      403         +         0
        Intr      481      885         +         0
        Term      969     1469         +         0

>2262097        /37663

      len =     1694    nex =        2

        Term    48814    47723         -         0
        Init    49413    49234         -         0

>2262097        /37704

      len =     1990    nex =        6

        Term     4521     4199         -         0
        Intr     4778     4665         -         0
        Intr     5379     5207         -         0
        Intr     5540     5489         -         0
        Intr     5680     5632         -         0
        Init     6186     5782         -         0

>2262097        /112955

      len =     2350    nex =        4

        Term    89371    88825         -         0
        Intr    89563    89456         -         0
        Intr    89803    89654         -         0
        Init    91172    90509         -         0

>2262135        /41490

      len =     1454    nex =        2

        Term     2318     1916         -         0
        Init     3369     2625         -         0

>2262135        /20167

      len =     1304    nex =        2

        Term     4241     3765         -         0
        Init     5068     4768         -         0

>2262135        /32291

      len =     1390    nex =        3

        Term     3887     3685         -         0
        Intr     4241     4100         -         0
        Init     5072     4768         -         0

>2262135        /6568

      len =     2212    nex =        6

        Term    55501    55152         -         0
        Intr    55716    55591         -         0
        Intr    55868    55793         -         0
```

```
     Intr   56088   55950         -        0
     Intr   56564   56483         -        0
     Init   57080   56653         -        0

>2262135        /10207

    len =     2063    nex =       4

     Init   59951   60024         +        0
     Intr   60681   60762         +        0
     Intr   61016   61098         +        0
     Term   61517   61813         +        0

>2262135        /18545

    len =      647    nex =       1

     Sngl    6145    6791         +        0

>2262135        /4346

    len =     2939    nex =       6

     Init   70603   71150         +        0
     Intr   71555   71677         +        0
     Intr   71842   71907         +        0
     Intr   71994   72059         +        0
     Intr   72734   72814         +        0
     Term   72893   73541         +        0

>2262135        /26127

    len =      817    nex =       1

     Sngl   10051   10199         +        0

>2262135        /8114

    len =     1879    nex =       4

     Init   97068   97416         +        0
     Intr   98158   98297         +        0
     Intr   98468   98540         +        0
     Term   98650   98946         +        0

>2262135        /34186

    len =      347    nex =       1

     Sngl   97069   97415         +        0

>2262135        /145375

    len =      319    nex =       1

     Sngl   10051   10164         +        0

>2262135        /18454

    len =      354    nex =       1
```

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```
    Sngl   10051   10199         +        0

>2262135       /27915

   len =     1173   nex =        3

    Init   99470   99712         +        0
    Intr   99822   99870         +        0
    Term   99982  100642         +        0

>2262155       /1441

   len =      657   nex =        1

    Sngl   23119   22463         -        0

>2262155       /38365

   len =     2443   nex =       11

    Term   33741   33609         -        0
    Intr   33874   33812         -        0
    Intr   34038   33961         -        0
    Intr   34207   34130         -        0
    Intr   34357   34283         -        0
    Intr   34542   34456         -        0
    Intr   35004   34864         -        0
    Intr   35174   35106         -        0
    Intr   35320   35254         -        0
    Intr   35536   35471         -        0
    Init   36051   35849         -        0

>2262155       /2578

   len =     2710   nex =       12

    Term   41819   41536         -        0
    Intr   42007   41945         -        0
    Intr   42177   42100         -        0
    Intr   42353   42276         -        0
    Intr   42507   42433         -        0
    Intr   42691   42605         -        0
    Intr   42920   42792         -        0
    Intr   43144   43004         -        0
    Intr   43300   43232         -        0
    Intr   43448   43382         -        0
    Intr   43690   43625         -        0
    Init   44238   44044         -        0

>2262155       /10042

   len =     1776   nex =        4

    Init   47118   47195         +        0
    Intr   47279   47459         +        0
    Intr   47575   47672         +        0
    Term   47837   48384         +        0

>2262155       /13246
```

09829525.062501

```
   len =    1990   nex =       6

    Init   54079   54165        +        0
    Intr   54255   54346        +        0
    Intr   54432   54540        +        0
    Intr   54640   54675        +        0
    Intr   54764   54850        +        0
    Term   54940   55113        +        0

>2262155        /34698

   len =    1459   nex =       6

    Init   56211   56260        +        0
    Intr   56344   56556        +        0
    Intr   56654   56802        +        0
    Intr   56878   57034        +        0
    Intr   57160   57252        +        0
    Term   57530   57669        +        0

>2262155        /39211

   len =    2110   nex =       2

    Init   64477   65546        +        0
    Term   66273   66579        +        0

>2262155        /19601

   len =    2050   nex =       2

    Init   64534   65546        +        0
    Term   66273   66579        +        0

>2262155        /32751

   len =     850   nex =       1

    Sngl   77445   76604        -        0

>2262155        /3276

   len =    1167   nex =       1

    Sngl    8628    9794        +        0

>2264302        /38370

   len =    1450   nex =       2

    Term   35101   34004        -        0
    Init   35452   35188        -        0

>2264302        /9562

   len =    2074   nex =       0

>2264302        /28046
```

```
    len =     1581   nex =        4

     Term   51719   51257         -          0
     Intr   52040   51910         -          0
     Intr   52474   52402         -          0
     Init   52837   52724         -          0

>2264302        /16428

    len =     1571   nex =        4

     Term   51818   51294         -          0
     Intr   52040   51910         -          0
     Intr   52474   52402         -          0
     Init   52864   52724         -          0

>2264302        /100085

    len =     1254   nex =        3

     Term    5287    4881         -          0
     Intr    5613    5357         -          0
     Init    6134    5782         -          0

>2264303        /22

    len =     1735   nex =        6

     Init   14289   14642         +          0
     Intr   14799   14910         +          0
     Intr   15002   15095         +          0
     Intr   15228   15405         +          0
     Intr   15488   15557         +          0
     Term   15638   16023         +          0

>2264303        /7145

    len =      824   nex =        4

     Init    3387    3465         +          0
     Intr    3544    3666         +          0
     Intr    3754    3870         +          0
     Term    3947    4205         +          0

>2264303        /4273

    len =     1845   nex =        3

     Term   45044   44650         -          0
     Intr   45266   45126         -          0
     Init   46494   46178         -          0

>2264303        /35612

    len =     1469   nex =        4

     Init   58748   59002         +          0
     Intr   59229   59277         +          0
     Intr   59634   59833         +          0
     Term   59930   60216         +          0
```

09555325.062600

```
>2264303        /42336

    len =     1825    nex =        4

     Term    64023    63682          -          0
     Intr    64570    64473          -          0
     Intr    65089    64989          -          0
     Init    65506    65289          -          0

>2264304        /34402

    len =     2558    nex =        5

     Init    20281    20902          +          0
     Intr    21285    21510          +          0
     Intr    21627    21849          +          0
     Intr    22104    22317          +          0
     Term    22554    22838          +          0

>2264304        /34783

    len =     2075    nex =        5

     Term    23983    23714          -          0
     Intr    24174    24080          -          0
     Intr    24709    24267          -          0
     Intr    25149    24793          -          0
     Init    25788    25400          -          0

>2264304        /39319

    len =     1870    nex =        5

     Init     2871     2989          +          0
     Intr     3690     3771          +          0
     Intr     3960     4165          +          0
     Intr     4328     4381          +          0
     Term     4476     4733          +          0

>2264304        /9159

    len =     1570    nex =        2

     Init    41803    42064          +          0
     Term    42974    43372          +          0

>2264304        /38464

    len =     1270    nex =        1

     Sngl    51034    52303          +          0

>2264304        /28578

    len =     2110    nex =        5

     Init      515     1139          +          0
     Intr     1407     1504          +          0
     Intr     1754     1853          +          0
```

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```
      Intr    2027    2272          +        0
      Term    2358    2618          +        0

    >2264304        /41195

     len =     353   nex =         1

      Sngl   57898   57549          -        0

    >2264304        /2871

     len =     430   nex =         2

      Init    6595    6647          +        0
      Term    6733    7019          +        0

    >2264304        /30073

     len =    1810   nex =         1

      Sngl   65320   65030          -        0

    >2264304        /32071

     len =    1128   nex =         1

      Sngl   67814   67283          -        0

    >2264304        /103464

     len =    1096   nex =         1

      Sngl   67814   67316          -        0

    >2264304        /17818

     len =    1136   nex =         1

      Sngl   67814   67277          -        0

    >2264304        /24095

     len =     596   nex =         1

      Sngl   72223   72818          +        0

    >2264304        /111741

     len =    2898   nex =         9

      Init   77610   77692          +        0
      Intr   78044   78153          +        0
      Intr   78600   78734          +        0
      Intr   78876   79022          +        0
      Intr   79400   79483          +        0
      Intr   79589   79635          +        0
      Intr   79729   79802          +        0
      Intr   79915   79973          +        0
      Term   80152   80212          +        0
```

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```
>2264305        /10263

   len =    1493   nex =       5

    Init   31119   31386          +        0
    Intr   31604   31784          +        0
    Intr   31864   32005          +        0
    Intr   32090   32159          +        0
    Term   32249   32611          +        0

>2264305        /98400

   len =     993   nex =       3

    Term    4415    4173          -        0
    Intr    4868    4742          -        0
    Init    5152    4965          -        0

>2264305        /36333

   len =    1450   nex =       4

    Term    4415    4119          -        0
    Intr    4868    4742          -        0
    Intr    5244    4965          -        0
    Init    5422    5374          -        0

>2264305        /121728

   len =     550   nex =       2

    Term    5244    5080          -        0
    Init    5422    5374          -        0

>2264305        /41072

   len =    1312   nex =       4

    Term    4415    4326          -        0
    Intr    4868    4742          -        0
    Intr    5244    4965          -        0
    Init    5422    5374          -        0

>2264305        /24983

   len =     599   nex =       1

    Sngl   64677   64079          -        0

>2264305        /16865

   len =    1615   nex =       4

    Init   71009   71096          +        0
    Intr   71447   71574          +        0
    Intr   71737   71841          +        0
    Term   72035   72347          +        0

>2264305        /35698
```

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```
      len =    1150   nex =       4

       Init   71025   71096       +        0
       Intr   71447   71574       +        0
       Intr   71737   71841       +        0
       Term   72035   72162       +        0

  >2264306       /21505

      len =    1450   nex =       3

       Term   10517   10132       -        0
       Intr   11048   10721       -        0
       Init   11577   11269       -        0

  >2264306       /19024

      len =     715   nex =       2

       Term   14439   14066       -        0
       Init   14777   14527       -        0

  >2264306       /33140

      len =    1450   nex =       3

       Term   14439   13966       -        0
       Intr   14854   14527       -        0
       Init   15411   14979       -        0

  >2264306       /121213

      len =     333   nex =       1

       Sngl    2596    2928       +        0

  >2264306       /39888

      len =    2203   nex =       9

       Term   35099   34644       -        0
       Intr   35279   35181       -        0
       Intr   35475   35371       -        0
       Intr   35651   35559       -        0
       Intr   35855   35763       -        0
       Intr   36011   35958       -        0
       Intr   36218   36117       -        0
       Intr   36369   36295       -        0
       Init   36846   36503       -        0

  >2264306       /11054

      len =    1417   nex =       5

       Init   41110   41228       +        0
       Intr   41333   41424       +        0
       Intr   41763   41818       +        0
       Intr   42120   42181       +        0
       Term   42324   42526       +        0
```

```
>2264306        /3699

   len =     1897    nex =        4

    Init     5030     5266          +         0
    Intr     5420     6238          +         0
    Intr     6325     6526          +         0
    Term     6551     6926          +         0

>2264306        /6637

   len =     1428    nex =        3

    Term    80382    79690          -         0
    Intr    80764    80484          -         0
    Init    81117    80852          -         0

>2264306        /111669

   len =      382    nex =        2

    Init    88535    88581          +         0
    Term    88664    88916          +         0

>2264307        /42441

   len =      682    nex =        2

    Term    48650    48344          -         0
    Init    49017    48966          -         0

>2264307        /22848

   len =      658    nex =        2

    Term    48650    48368          -         0
    Init    49017    48966          -         0

>2264307        /145394

   len =      638    nex =        2

    Term    48650    48388          -         0
    Init    49017    48966          -         0

>2264307        /11511

   len =      776    nex =        2

    Term    48650    48252          -         0
    Init    49027    48966          -         0

>2264307        /12330

   len =      670    nex =        2

    Term    48650    48363          -         0
    Init    49017    48966          -         0

>2264307        /37668
```

```
     len =     2959    nex =       12

      Term    58676    58435          -         0
      Intr    58819    58762          -         0
      Intr    59006    58939          -         0
      Intr    59148    59089          -         0
      Intr    59415    59374          -         0
      Intr    59547    59504          -         0
      Intr    59753    59684          -         0
      Intr    60223    60104          -         0
      Intr    60499    60481          -         0
      Intr    60688    60616          -         0
      Intr    60911    60847          -         0
      Init    61393    61056          -         0

 >2264307         /24058

     len =     1653    nex =        4

      Init    72492    72816          +         0
      Intr    73287    73411          +         0
      Intr    73485    73593          +         0
      Term    73888    74144          +         0

 >2264308         /1935

     len =     1396    nex =        1

      Sngl    17599    16204          -         0

 >2264308         /22483

     len =     2981    nex =        8

      Term     4792     4416          -         0
      Intr     5296     4866          -         0
      Intr     5495     5375          -         0
      Intr     5737     5588          -         0
      Intr     6028     5823          -         0
      Intr     6224     6110          -         0
      Intr     6544     6307          -         0
      Init     7396     7131          -         0

 >2264309         /37959

     len =      357    nex =        1

      Sngl    16800    16444          -         0

 >2264309         /15155

     len =      872    nex =        2

      Init    22581    22830          +         0
      Term    22927    23337          +         0

 >2264309         /36334

     len =     4030    nex =        8
```

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```
    Term   23729   23461       -      0
    Intr   23957   23827       -      0
    Intr   24155   24049       -      0
    Intr   24319   24241       -      0
    Intr   24499   24413       -      0
    Intr   26484   26236       -      0
    Intr   26721   26572       -      0
    Init   27488   26913       -      0

>2264309       /109246

   len =     614   nex =      1

    Sngl   36598   37211       +      0

>2264309       /34868

   len =    2755   nex =     10

    Init   56456   56771       +      0
    Intr   57170   57262       +      0
    Intr   57346   57427       +      0
    Intr   57612   57708       +      0
    Intr   57802   57877       +      0
    Intr   58009   58067       +      0
    Intr   58236   58358       +      0
    Intr   58523   58580       +      0
    Intr   58667   58752       +      0
    Term   58834   59210       +      0

>2264310       /99461

   len =     692   nex =      1

    Sngl   11215   11906       +      0

>2264310       /15761

   len =    2548   nex =      6

    Term   19001   18686       -      0
    Intr   19291   19099       -      0
    Intr   19675   19440       -      0
    Intr   19965   19793       -      0
    Intr   20557   20507       -      0
    Init   21233   20635       -      0

>2264310       /11083

   len =     565   nex =      1

    Sngl    2390    2039       -      0

>2264310       /31527

   len =     589   nex =      1

    Sngl   45291   45879       +      0
```

```
>2264310       /17408

   len =     642   nex =       1

    Sngl   75188   74547         -        0

>2264310       /125083

   len =    1961   nex =       5

    Init    8184    8440         +        0
    Intr    8574    8786         +        0
    Intr    8879    9037         +        0
    Intr    9616    9684         +        0
    Term    9797   10144         +        0

>2264311       /32868

   len =    1724   nex =       5

    Term   22845   22268         -        0
    Intr   23036   22924         -        0
    Intr   23230   23115         -        0
    Intr   23684   23307         -        0
    Init   23977   23868         -        0

>2264311       /6256

   len =     970   nex =       3

    Term   61688   61655         -        0
    Intr   61915   61777         -        0
    Init   62223   62000         -        0

>2264311       /125951

   len =    2213   nex =       8

    Term   60708   60456         -        0
    Intr   60920   60814         -        0
    Intr   61074   61009         -        0
    Intr   61491   61410         -        0
    Intr   61688   61644         -        0
    Intr   61915   61777         -        0
    Intr   62223   62000         -        0
    Init   62668   62430         -        0

>2264311       /27195

   len =    1880   nex =       7

    Term   82920   82401         -        0
    Intr   83150   83009         -        0
    Intr   83482   83243         -        0
    Intr   83616   83581         -        0
    Intr   83788   83708         -        0
    Intr   83928   83871         -        0
    Init   84280   84011         -        0

>2264312       /14950
```

```
     len =      881    nex =        1

      Sngl   27808   26928          -         0

>2264312        /95433

     len =     1318    nex =        5

      Term   41828   41661          -         0
      Intr   42031   41958          -         0
      Intr   42285   42119          -         0
      Intr   42519   42450          -         0
      Init   42741   42601          -         0

>2264312        /41937

     len =      412    nex =        1

      Sngl   46315   45915          -         0

>2264312        /13715

     len =      505    nex =        1

      Sngl   46419   45915          -         0

>2264312        /20908

     len =     1588    nex =        1

      Sngl   47047   45915          -         0

>2264312        /121153

     len =     1599    nex =        0

>2264312        /21872

     len =     1999    nex =        5

      Init   76178   76439          +         0
      Intr   76875   77278          +         0
      Intr   77349   77609          +         0
      Intr   77680   77802          +         0
      Term   77884   78176          +         0

>2264312        /40252

     len =      929    nex =        3

      Init    8129    8281          +         0
      Intr    8374    8529          +         0
      Term    8834    9057          +         0

>2264313        /13012

     len =     2530    nex =        3

      Init   50735   51416          +         0
     Intr    51723   52053          +        0
     Term    52969   53262          +        0

>2264313        /156373

   len =     1597    nex =        4

     Term    56197   55946          -        0
     Intr    56442   56319          -        0
     Intr    57210   56988          -        0
     Init    57542   57464          -        0

>2264314        /8635

   len =     1886    nex =        4

     Term    10067    9103          -        0
     Intr    10250   10148          -        0
     Intr    10433   10340          -        0
     Init    10988   10835          -        0

>2264314        /115644

   len =     1259    nex =        2

     Term    26540   26126          -        0
     Init    27384   26837          -        0

>2264314        /38996

   len =     2313    nex =        7

     Term    27833   27526          -        0
     Intr    28049   27984          -        0
     Intr    28349   28278          -        0
     Intr    28813   28492          -        0
     Intr    29046   28886          -        0
     Intr    29175   29131          -        0
     Init    29838   29580          -        0

>2264314        /32785

   len =     1499    nex =        1

     Sngl    41738   42167          +        0

>2264314        /20245

   len =     1429    nex =        1

     Sngl    41738   42147          +        0

>2264314        /5592

   len =     1450    nex =        0

>2264314        /13819

   len =     1390    nex =        1
```

```
     Sngl    41738    42167         +         0

>2264314        /29726

     len =      673    nex =        1

     Sngl    46055    46727         +         0

>2264314        /41900

     len =      567    nex =        1

     Sngl    46131    46697         +         0

>2264314        /2462

     len =      570    nex =        1

     Sngl    46131    46700         +         0

>2264314        /16750

     len =      585    nex =        1

     Sngl    46131    46715         +         0

>2264314        /18232

     len =     1571    nex =        5

      Term    48315    47879         -         0
      Intr    48456    48413         -         0
      Intr    48598    48541         -         0
      Intr    48919    48826         -         0
      Init    49449    49182         -         0

>2264314        /9012

     len =     1870    nex =        0

>2264314        /7365

     len =     1776    nex =        0

>2264314        /33059

     len =     2811    nex =        7

      Term    61633    61320         -         0
      Intr    61973    61823         -         0
      Intr    62227    62054         -         0
      Intr    62409    62320         -         0
      Intr    62646    62576         -         0
      Intr    63811    62772         -         0
      Init    64130    63836         -         0

>2264314        /27647

     len =     1370    nex =        3
     Init    72212    72591          +          0
     Intr    72849    73086          +          0
     Term    73196    73581          +          0

>2264315          /10218

   len =      2270    nex =        4

     Term    26015    25438          -          0
     Intr    26141    26094          -          0
     Intr    27175    26240          -          0
     Init    27707    27384          -          0
>2264315          /29462

   len =      1139    nex =        2

     Init    45117    45873          +          0
     Term    45961    46255          +          0

>2264315          /14965

   len =       430    nex =        1

     Sngl    47036    46610          -          0

>2264315          /114307

   len =       464    nex =        1

     Sngl    47105    46642          -          0

>2264315          /3363

   len =       636    nex =        1

     Sngl    47111    46476          -          0

>2264315          /41666

   len =      2157    nex =       12

     Init    59476    59703          +          0
     Intr    59800    59887          +          0
     Intr    60015    60074          +          0
     Intr    60160    60192          +          0
     Intr    60278    60355          +          0
     Intr    60433    60476          +          0
     Intr    60582    60622          +          0
     Intr    60709    60791          +          0
     Intr    60876    60967          +          0
     Intr    61055    61124          +          0
     Intr    61205    61246          +          0
     Term    61348    61632          +          0

>2264316          /31759

   len =      1810    nex =        3

     Term    40887    40024          -          0
     Intr    41245    40976          -          0
     Init   41826   41375          -        0

>2264316       /4716

   len =     1150   nex =        4

     Term   48078   47771          -        0
     Intr   48347   48169          -        0
     Intr   48549   48448          -        0
     Init   48918   48760          -        0

>2264316       /35357

   len =     3430   nex =        2

     Init    4937    5508          +        0
     Term    7116    8360          +        0

>2264316       /13418

   len =     1121   nex =        4

     Term   50134   49841          -        0
     Intr   50452   50271          -        0
     Intr   50665   50567          -        0
     Init   50961   50832          -        0

>2264316       /25839

   len =     1733   nex =        4

     Term   52037   51717          -        0
     Intr   52799   52621          -        0
     Intr   52994   52893          -        0
     Init   53449   53248          -        0

>2264316       /5103

   len =      566   nex =        2

     Term   56108   55749          -        0
     Init   56314   56188          -        0

>2264316       /25723

   len =     1118   nex =        3

     Init   70502   70609          +        0
     Intr   70687   70765          +        0
     Term   71265   71619          +        0

>2264316       /28686

   len =     1761   nex =        5

     Init   73159   73478          +        0
     Intr   73823   73864          +        0
     Intr   74151   74238          +        0
     Intr   74355   74436          +        0
     Term   74532   74919          +        0
```

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```
>2264316        /33187

   len =     1316    nex =       6

     Init   75294    75411          +         0
     Intr   75493    75533          +         0
     Intr   75623    75723          +         0
     Intr   75977    76121          +         0
     Intr   76215    76304          +         0
     Term   76389    76609          +         0

>2264316        /40559

   len =      940    nex =       4

     Init   75623    75723          +         0
     Intr   75977    76121          +         0
     Intr   76215    76304          +         0
     Term   76389    76430          +         0

>2264317        /27304

   len =     1450    nex =       4

     Init   10536    10865          +         0
     Intr   11094    11307          +         0
     Intr   11430    11575          +         0
     Term   11678    11977          +         0

>2264317        /41386

   len =     2230    nex =       7

     Init   18624    18806          +         0
     Intr   19320    19433          +         0
     Intr   19544    19688          +         0
     Intr   19786    19863          +         0
     Intr   19964    20076          +         0
     Intr   20166    20269          +         0
     Term   20364    20848          +         0

>2264317        /19638

   len =     1116    nex =       5

     Term   39626    39380          -         0
     Intr   39837    39741          -         0
     Intr   39994    39932          -         0
     Intr   40263    40110          -         0
     Init   40495    40353          -         0

>2264317        /6734

   len =     2230    nex =       8

     Init   43041    43121          +         0
     Intr   43615    43732          +         0
     Intr   43820    43927          +         0
     Intr   44029    44153          +         0
```

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```
    Intr   44256   44520          +        0
    Intr   44612   44680          +        0
    Intr   44773   44934          +        0
    Term   45031   45269          +        0

>2264318       /3797

  len =    3010   nex =      11

    Term   14549   14209          -        0
    Intr   14698   14642          -        0
    Intr   14911   14777          -        0
    Intr   15084   15004          -        0
    Intr   15230   15162          -        0
    Intr   15408   15334          -        0
    Intr   15837   15757          -        0
    Intr   16050   15932          -        0
    Intr   16304   16139          -        0
    Intr   16522   16393          -        0
    Init   17210   16609          -        0

>2264318       /33231

  len =    1510   nex =       4

    Term   19006   18683          -        0
    Intr   19387   19102          -        0
    Intr   19635   19485          -        0
    Init   20191   19835          -        0

>2264318       /42276

  len =     681   nex =       1

    Sngl   24794   24114          -        0

>2264318       /26752

  len =     754   nex =       1

    Sngl    6372    6627          +        0

>2264318       /25855

  len =    2410   nex =       4

    Init   74093   74435          +        0
    Intr   74770   74907          +        0
    Intr   75288   75359          +        0
    Term   75730   76502          +        0

>2264319       /37985

  len =    1041   nex =       3

    Term   29497   28961          -        0
    Intr   29867   29820          -        0
    Init   30001   29945          -        0

>2264320       /36697
```

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```
     len =    3070   nex =      12

      Init   77774    77917        +        0
      Intr   78577    78735        +        0
      Intr   78827    78886        +        0
      Intr   79001    79047        +        0
      Intr   79159    79212        +        0
      Intr   79302    79479        +        0
      Intr   79602    79754        +        0
      Intr   79848    79913        +        0
      Intr   80000    80047        +        0
      Intr   80127    80233        +        0
      Intr   80327    80405        +        0
      Term   80513    80843        +        0

 >2264321       /22350

     len =    1378   nex =       1

      Sngl   42485    43862        +        0

 >2264321       /17195

     len =    2639   nex =       9

      Term   44138    43885        -        0
      Intr   44545    44459        -        0
      Intr   44723    44638        -        0
      Intr   45000    44910        -        0
      Intr   45164    45079        -        0
      Intr   45369    45261        -        0
      Intr   45565    45519        -        0
      Intr   45725    45656        -        0
      Init   46523    45984        -        0

 >2264321       /4025

     len =    1845   nex =       8

      Term   62897    62608        -        0
      Intr   63027    62981        -        0
      Intr   63329    63108        -        0
      Intr   63461    63409        -        0
      Intr   63720    63555        -        0
      Intr   63853    63812        -        0
      Intr   64024    63940        -        0
      Init   64452    64119        -        0

 >2264321       /226

     len =    1214   nex =       3

      Init   64562    64824        +        0
      Intr   65227    65327        +        0
      Term   65506    65775        +        0

 >2264321       /25843

     len =    1179   nex =       1
     Sngl    64602   65780          +         0

>2264367        /13226

    len =      760   nex =          1

     Sngl    17702   16945          -         0

>2264367        /6280

    len =     1721   nex =          4

     Init    79635   80401          +         0
     Intr    80649   80739          +         0
     Intr    80875   81047          +         0
     Term    81136   81355          +         0

>2264367        /14253

    len =      394   nex =          1

     Sngl    79694   80087          +         0

>2264367        /2093

    len =     1697   nex =          4

     Term    81924   81450          -         0
     Intr    82092   82014          -         0
     Intr    82411   82172          -         0
     Init    83146   82545          -         0

>2275194       `/35109

    len =     1541   nex =          0

>2275194        /20378

    len =      540   nex =          1

     Sngl    46427   45888          -         0

>2275194        /6324

    len =      564   nex =          1

     Sngl    81129   81692          +         0

>2275194        /95662

    len =      550   nex =          0

>2275194        /21715

    len =      339   nex =          1

     Sngl    81340   81678          +         0

>2275194        /34414
```

```
      len =     2177    nex =        6

        Init     1529     1687          +          0
        Intr     1807     1877          +          0
        Intr     2195     2314          +          0
        Intr     2406     2524          +          0
        Intr     2616     2697          +          0
        Term     2789     3076          +          0

  >2275194         /35584

      len =     2064    nex =        6

        Init     1529     1687          +          0
        Intr     1807     1877          +          0
        Intr     2195     2314          +          0
        Intr     2406     2524          +          0
        Intr     2616     2697          +          0
        Term     2789     3017          +          0

  >2281081         /99937

      len =     1179    nex =        4

        Init    17994    18277          +          0
        Intr    18570    18617          +          0
        Intr    18757    18836          +          0
        Term    18973    19172          +          0

  >2281081         /34407

      len =     1614    nex =        2

        Term    20892    20042          -          0
        Init    21655    20980          -          0

  >2281081         /24415

      len =     1398    nex =        1

        Sngl    37217    37695          +          0

  >2281081         /21866

      len =     3043    nex =       10

        Init    41405    41802          +          0
        Intr    41989    42098          +          0
        Intr    42186    42243          +          0
        Intr    42347    42610          +          0
        Intr    42881    43018          +          0
        Intr    43151    43202          +          0
        Intr    43288    43367          +          0
        Intr    43475    43534          +          0
        Intr    43663    43743          +          0
        Term    44186    44447          +          0

  >2281081         /117763
```

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```
     len =      168    nex =        1

      Sngl   44280   44447         +         0

>2281081        /37969

     len =     1630    nex =        2

      Init   45636   46252         +         0
      Term   46437   47256         +         0

>2281081        /97249

     len =     1570    nex =        3

      Init   75474   75567         +         0
      Intr   75664   75773         +         0
      Term   76110   76381         +         0

>2288979        /30737

     len =     1957    nex =        6

      Term   23749   23549         -         0
      Intr   24382   24215         -         0
      Intr   24583   24465         -         0
      Intr   24734   24673         -         0
      Intr   24906   24830         -         0
      Init   25505   25278         -         0

>2288979        /42038

     len =     2417    nex =        6

      Term   26123   25700         -         0
      Intr   26352   26213         -         0
      Intr   26728   26523         -         0
      Intr   27113   27007         -         0
      Intr   27509   27330         -         0
      Init   28116   27832         -         0

>2288979        /5460

     len =     1369    nex =        2

      Term   61213   60939         -         0
      Init   61831   61648         -         0

>2288979        /31535

     len =      971    nex =        1

      Sngl    6953    5983         -         0

>2288979        /15927

     len =      582    nex =        2

      Init   83467   83577         +         0
      Term   83732   84048         +         0
```

```
>2288979        /14769

   len =      598   nex =        2

    Init   84968   85307         +         0
    Term   85318   85559         +         0

>2288979        /22360

   len =      621   nex =        3

    Init   84968   85076         +         0
    Intr   85239   85307         +         0
    Term   85318   85582         +         0

>2288979        /8155

   len =      637   nex =        3

    Init   84968   85076         +         0
    Intr   85239   85307         +         0
    Term   85318   85598         +         0

>2288979        /91704

   len =      685   nex =        2

    Init   85879   86099         +         0
    Term   86237   86563         +         0

>2288979        /14241

   len =      594   nex =        2

    Init   85971   86099         +         0
    Term   86237   86564         +         0

>2288979        /27364

   len =      593   nex =        1

    Sngl   87277   87869         +         0

>2288979        /16079

   len =      558   nex =        2

    Init   88140   88265         +         0
    Term   88343   88697         +         0

>2288979        /85

   len =      670   nex =        2

    Init   88140   88265         +         0
    Term   88343   88809         +         0

>2288979        /35036
```

```
      len =      510    nex =         2

       Term   89987    89866          -          0
       Init   90375    90255          -          0

  >2326340        /17730

      len =      938    nex =         3

       Init   12848    12929          +          0
       Intr   13222    13294          +          0
       Term   13456    13785          +          0

  >2335089        /17415

      len =     1711    nex =         2

       Init   18997    19833          +          0
       Term   20359    20707          +          0

  >2335089        /41462

      len =     2561    nex =         7

       Init   77553    77859          +          0
       Intr   78200    78282          +          0
       Intr   78527    78615          +          0
       Intr   78796    78869          +          0
       Intr   78950    79000          +          0
       Intr   79347    79408          +          0
       Term   79492    80113          +          0

  >2337888        /30632

      len =      597    nex =         1

       Sngl   45399    44803          -          0

  >2337888        /33132

      len =     1427    nex =         1

       Sngl   56372    54946          -          0

  >2337888        /25271

      len =     1190    nex =         4

       Term   81979    81460          -          0
       Intr   82251    82069          -          0
       Intr   82443    82348          -          0
       Init   82649    82529          -          0

  >2337888        /36364

      len =     2473    nex =        10

       Term   81979    81474          -          0
       Intr   82251    82069          -          0
       Intr   82443    82348          -          0
      Intr    82588    82529          -        0
      Intr    82726    82673          -        0
      Intr    82906    82829          -        0
      Intr    83042    82989          -        0
      Intr    83230    83147          -        0
      Intr    83655    83627          -        0
      Init    83946    83768          -        0

>2337888         /48

     len =      139    nex =         1

      Sngl    84105    83967          -        0

>2337888         /39291

     len =     1330    nex =         2

      Init     9724    10277          +        0
      Term    10380    11048          +        0

>2341023         /20848

     len =     2394    nex =         8

      Term   105381   105134          -        0
      Intr   105770   105531          -        0
      Intr   106011   105948          -        0
      Intr   106356   106242          -        0
      Intr   106669   106531          -        0
      Intr   106971   106841          -        0
      Intr   107209   107080          -        0
      Init   107527   107476          -        0

>2341023         /4513

     len =     1287    nex =         3

      Term    16071    15822          -        0
      Intr    16960    16676          -        0
      Init    17108    17082          -        0

>2341023         /26558

     len =     1150    nex =         3

      Term    23857    23331          -        0
      Intr    24045    23945          -        0
      Init    24472    24392          -        0

>2341023         /23398

     len =     2892    nex =         2

      Term    36567    36137          -        0
      Init    39028    38927          -        0

>2341023         /40467

     len =     2202    nex =         7
     Init   41815   41979         +         0
     Intr   42299   42457         +         0
     Intr   42564   42739         +         0
     Intr   42897   43174         +         0
     Intr   43264   43399         +         0
     Intr   43492   43603         +         0
     Term   43692   44016         +         0

>2341023        /19832

    len =    2656   nex =        4

     Init   45198   45615         +         0
     Intr   45720   45944         +         0
     Intr   46040   46752         +         0
     Term   46898   47342         +         0

>2341023        /91880

    len =    1118   nex =        2

     Init   46306   46752         +         0
     Term   46898   47423         +         0

>2341023        /8374

    len =     805   nex =        3

     Init   84788   85031         +         0
     Intr   85113   85256         +         0
     Term   85340   85592         +         0

>2341023        /9471

    len =     649   nex =        1

     Sngl   85423   85236         -         0

>2341023        /30909

    len =    1020   nex =        3

     Init   90351   90483         +         0
     Intr   90571   90628         +         0
     Term   91104   91353         +         0

>2341023        /28606

    len =     730   nex =        1

     Sngl   91839   92568         +         0

>2341023        /125151

    len =     310   nex =        1

     Sngl   96904   96600         -         0

>2341023        /33613
```

```
   len =    2290   nex =       5

    Term   94901   94658       -         0
    Intr   95464   95403       -         0
    Intr   95744   95606       -         0
    Intr   96270   96059       -         0
    Init   96946   96584       -         0

>2342673        /21644

   len =     568   nex =       2

    Init       1      19       +         0
    Term     287     568       +         0

>2342673        /4236

   len =    1031   nex =       1

    Sngl   15499   14469       -         0

>2342673        /13218

   len =     600   nex =       1

    Sngl   59777   59178       -         0

>2342673        /1911

   len =    1410   nex =       0

>2342673        /15745

   len =    2693   nex =      15

    Term   72951   72598       -         0
    Intr   73173   73059       -         0
    Intr   73327   73268       -         0
    Intr   73473   73420       -         0
    Intr   73651   73592       -         0
    Intr   73809   73747       -         0
    Intr   73936   73893       -         0
    Intr   74109   74025       -         0
    Intr   74283   74203       -         0
    Intr   74471   74379       -         0
    Intr   74618   74554       -         0
    Intr   74789   74714       -         0
    Intr   74956   74891       -         0
    Intr   75176   75051       -         0
    Init   75290   75255       -         0

>2342673        /20814

   len =    2669   nex =      14

    Term   87698   87414       -         0
    Intr   87906   87792       -         0
    Intr   88057   87998       -         0
    Intr   88219   88166       -         0
```

```
       Intr   88375   88316         -         0
       Intr   88529   88467         -         0
       Intr   88664   88621         -         0
       Intr   88853   88769         -         0
       Intr   89044   88964         -         0
       Intr   89241   89149         -         0
       Intr   89408   89344         -         0
       Intr   89583   89508         -         0
       Intr   89751   89686         -         0
       Init   89916   89851         -         0

>2342673       /36585

     len =    3206   nex =      16

       Term   87698   87522         -         0
       Intr   87906   87792         -         0
       Intr   88057   87998         -         0
       Intr   88219   88166         -         0
       Intr   88375   88316         -         0
       Intr   88529   88467         -         0
       Intr   88664   88621         -         0
       Intr   88853   88769         -         0
       Intr   89044   88964         -         0
       Intr   89241   89149         -         0
       Intr   89408   89344         -         0
       Intr   89583   89508         -         0
       Intr   89751   89686         -         0
       Intr   89916   89851         -         0
       Intr   90281   90192         -         0
       Init   90727   90584         -         0

>2342673       /39667

     len =     827   nex =       2

       Init   95406   95717         +         0
       Term   95822   96232         +         0

>2342717       /13928

     len =    4710   nex =      16

       Term   28916   28495         -         0
       Intr   29102   29002         -         0
       Intr   29276   29211         -         0
       Intr   29479   29365         -         0
       Intr   29760   29654         -         0
       Intr   29937   29848         -         0
       Intr   30204   30094         -         0
       Intr   30570   30505         -         0
       Intr   30730   30665         -         0
       Intr   31414   31265         -         0
       Intr   31587   31513         -         0
       Intr   32170   32079         -         0
       Intr   32332   32267         -         0
       Intr   32516   32417         -         0
       Intr   32772   32611         -         0
       Init   33012   32912         -         0
```

```
>2342717        /23892

   len =    1550   nex =       4

    Term   33902   33442        -        0
    Intr   34398   34340        -        0
    Intr   34564   34485        -        0
    Init   34991   34651        -        0

>2342717        /25519

   len =    2805   nex =       5

    Term   38674   38181        -        0
    Intr   38927   38769        -        0
    Intr   39218   39037        -        0
    Intr   40474   40303        -        0
    Init   40985   40560        -        0

>2351061        /36048

   len =    2257   nex =       4

    Term   36654   36150        -        0
    Intr   37353   37320        -        0
    Intr   37883   37644        -        0
    Init   38406   38255        -        0

>2351061        /16286

   len =    1302   nex =       2

    Init   60023   60178        +        0
    Term   60434   60780        +        0

>2351061        /25119

   len =    2152   nex =       5

    Init   72312   72460        +        0
    Intr   72978   73443        +        0
    Intr   73577   73670        +        0
    Intr   73763   73893        +        0
    Term   74106   74463        +        0

>2351061        /7022

   len =    1348   nex =       1

    Sngl   74769   74513        -        0

>2351061        /37512

   len =    1737   nex =       0

>2351062        /1575

   len =    1492   nex =       2

    Init   11143   11366        +        0
```

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```
      Term    11952    12270         +         0

   >2351062        /38092

      len =     2470    nex =        3

      Term    27085    26904         -         0
      Intr    28828    27521         -         0
      Init    29365    29247         -         0

   >2351062        /17241

      len =     1404    nex =        3

      Init    29965    30040         +         0
      Intr    30233    30463         +         0
      Term    30712    30955         +         0

   >2351062        /31041

      len =     2710    nex =        8

      Init    50901    51179         +         0
      Intr    51563    51664         +         0
      Intr    51779    51832         +         0
      Intr    52010    52102         +         0
      Intr    52264    52356         +         0
      Intr    52687    52791         +         0
      Intr    52881    52979         +         0
      Term    53072    53603         +         0

   >2351062        /23924

      len =     1277    nex =        3

      Init    71481    71998         +         0
      Intr    72070    72397         +         0
      Term    72483    72757         +         0

   >2351063        /114691

      len =     1789    nex =        8

      Term    20785    20575         -         0
      Intr    20954    20889         -         0
      Intr    21132    21047         -         0
      Intr    21269    21235         -         0
      Intr    21455    21369         -         0
      Intr    21616    21539         -         0
      Intr    21741    21701         -         0
      Init    22363    22239         -         0

   >2351063        /36626

      len =     1476    nex =        6

      Term    21132    21053         -         0
      Intr    21269    21235         -         0
      Intr    21455    21369         -         0
      Intr    21616    21539         -         0
      Intr   21741   21701         -        0
      Init   22528   22239         -        0

    >2351063       /31913

     len =    1211    nex =       4

      Init   28196   28319         +        0
      Intr   28394   28464         +        0
      Intr   28552   28573         +        0
      Term   28658   29015         +        0

    >2351063       /103246

     len =    1195    nex =       4

      Init   28196   28319         +        0
      Intr   28394   28464         +        0
      Intr   28552   28573         +        0
      Term   28658   29015         +        0

    >2351063       /36058

     len =    2835    nex =      10

      Init   55242   55559         +        0
      Intr   55634   55699         +        0
      Intr   55825   55890         +        0
      Intr   56186   56264         +        0
      Intr   56488   56608         +        0
      Intr   56694   56789         +        0
      Intr   56864   56976         +        0
      Intr   57238   57354         +        0
      Intr   57635   57735         +        0
      Term   57871   58076         +        0

    >2351063       /95281

     len =     430    nex =       1

      Sngl   58996   59416         +        0

    >2351063       /108981

     len =     314    nex =       1

      Sngl   62819   63132         +        0

    >2351063       /19716

     len =    2088    nex =       8

      Term   66456   66175         -        0
      Intr   66816   66527         -        0
      Intr   67192   66895         -        0
      Intr   67350   67280         -        0
      Intr   67560   67444         -        0
      Intr   67709   67635         -        0
      Intr   67857   67796         -        0
      Init   68262   68028         -        0
```

```
>2351063        /18140

  len =    3071    nex =       5

   Term   81242    80797        -         0
   Intr   81474    81378        -         0
   Intr   81610    81555        -         0
   Intr   81979    81686        -         0
   Init   82808    82071        -         0

>2351064        /10154

  len =    1092    nex =       5

   Init   30526    30610        +         0
   Intr   30871    30941        +         0
   Intr   31032    31188        +         0
   Intr   31364    31450        +         0
   Term   31536    31617        +         0

>2351064        /23922

  len =    2156    nex =       9

   Init   30531    30610        +         0
   Intr   30871    30941        +         0
   Intr   31032    31188        +         0
   Intr   31364    31450        +         0
   Intr   31536    31687        +         0
   Intr   31802    31882        +         0
   Intr   31983    32091        +         0
   Intr   32233    32359        +         0
   Term   32454    32686        +         0

>2351064        /41054

  len =     500    nex =       2

   Init   32229    32359        +         0
   Term   32454    32728        +         0

>2351064        /37122

  len =    2271    nex =       5

   Term   52016    51678        -         0
   Intr   52304    52104        -         0
   Intr   52616    52417        -         0
   Intr   52811    52698        -         0
   Init   53187    53050        -         0

>2351065        /8508

  len =     286    nex =       1

   Sngl    1156      871        -         0

>2351065        /29363
```

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```
    len =     8125    nex =         3

     Term     5274     4953        -          0
     Intr    12650     5804        -          0
     Init    13070    12743        -          0

>2351065         /3542

    len =     1606    nex =         3

     Term    12650    12382        -          0
     Intr    13557    12743        -          0
     Init    13987    13679        -          0

>2351065         /117588

    len =     1433    nex =         3

     Init    26825    26985        +          0
     Intr    27076    27149        +          0
     Term    27414    28257        +          0

>2351065         /15229

    len =     1952    nex =         9

     Term    28953    28676        -          0
     Intr    29086    29035        -          0
     Intr    29404    29169        -          0
     Intr    29662    29605        -          0
     Intr    29821    29753        -          0
     Intr    30022    29914        -          0
     Intr    30232    30165        -          0
     Intr    30434    30315        -          0
     Init    30627    30561        -          0

>2351065         /41047

    len =     1956    nex =         9

     Term    28953    28675        -          0
     Intr    29086    29035        -          0
     Intr    29404    29169        -          0
     Intr    29662    29605        -          0
     Intr    29821    29753        -          0
     Intr    30022    29914        -          0
     Intr    30232    30165        -          0
     Intr    30434    30315        -          0
     Init    30630    30561        -          0

>2351065         /105944

    len =      254    nex =         1

     Sngl    38996    38743        -          0

>2351065         /6823

    len =      436    nex =         1
```

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```
        Sngl      420       855          +          0

    >2351065         /15640

        len =      2139     nex =        7

        Term     54303     53997         -          0
        Intr     54528     54415         -          0
        Intr     54773     54648         -          0
        Intr     55027     54948         -          0
        Intr     55198     55117         -          0
        Intr     55390     55316         -          0
        Init     56135     55791         -          0

    >2351065         /633

        len =       529     nex =        1

        Sngl     56522     57050         +          0

    >2351065         /104017

        len =      1017     nex =        2

        Term     62259     61832         -          0
        Init     62503     62277         -          0

    >2351066         /92216

        len =      1063     nex =        1

        Sngl      2252      1951         -          0

    >2351066         /18332

        len =       372     nex =        1

        Sngl     51067     50696         -          0

    >2351066         /19255

        len =      1001     nex =        2

        Init      6275      6505         +          0
        Term      6677      6809         +          0

    >2351066         /93148

        len =       557     nex =        1

        Sngl     64963     64407         -          0

    >2351066         /9184

        len =      1493     nex =        3

        Init     65437     65484         +          0
        Intr     65563     65622         +          0
        Term     66328     66800         +          0
```

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```
>2351066        /94924

   len =      772    nex =        4

    Term   66989    66757        -         0
    Intr   67176    67069        -         0
    Intr   67314    67274        -         0
    Init   67528    67424        -         0

>2351066        /117503

   len =     1270    nex =        5

    Term   82968    82813        -         0
    Intr   83338    83123        -         0
    Intr   83553    83453        -         0
    Intr   83928    83699        -         0
    Init   84064    83998        -         0

>2351067        /24137

   len =      592    nex =        1

    Sngl   23773    23182        -         0

>2351067        /102435

   len =     1553    nex =        1

    Sngl   31407    31589        +         0

>2351067        /42506

   len =      913    nex =        2

    Init    3624     3761        +         0
    Term    4109     4536        +         0

>2351067        /37503

   len =     1398    nex =        4

    Term   39519    39286        -         0
    Intr   39736    39638        -         0
    Intr   40371    40283        -         0
    Init   40683    40599        -         0

>2351067        /23800

   len =     1450    nex =        4

    Term   39519    39294        -         0
    Intr   39736    39638        -         0
    Intr   40371    40283        -         0
    Init   40735    40599        -         0

>2351067        /12458

   len =      192    nex =        1
   Sngl   43705   43896         +         0

>2351068        /108814

   len =      755   nex =        4

   Init   14299   14392         +         0
   Intr   14508   14644         +         0
   Intr   14771   14817         +         0
   Term   14906   15053         +         0

>2351068        /33315

   len =     2311   nex =        9

   Init   14309   14392         +         0
   Intr   14508   14644         +         0
   Intr   14771   14817         +         0
   Intr   14906   15231         +         0
   Intr   15511   15593         +         0
   Intr   15693   15768         +         0
   Intr   15855   16012         +         0
   Intr   16102   16263         +         0
   Term   16357   16619         +         0

>2351068        /37265

   len =     2272   nex =        9

   Init   14347   14392         +         0
   Intr   14508   14644         +         0
   Intr   14771   14817         +         0
   Intr   14906   15231         +         0
   Intr   15511   15593         +         0
   Intr   15693   15768         +         0
   Intr   15855   16012         +         0
   Intr   16102   16263         +         0
   Term   16357   16618         +         0

>2351068        /777

   len =      540   nex =        1

   Sngl   22901   23440         +         0

>2351068        /2304

   len =      550   nex =        1

   Sngl   22901   23442         +         0

>2351068        /15211

   len =      560   nex =        1

   Sngl   22904   23463         +         0

>2351068        /27372

   len =     1870   nex =        3
```

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```
      Init    42505    42957         +         0
      Intr    43205    43414         +         0
      Term    43963    44371         +         0

 >2351068        /5335

    len =      731    nex =        3

      Init     5218     5304         +         0
      Intr     5320     5477         +         0
      Term     5551     5931         +         0

 >2351068        /22794

    len =      857    nex =        1

      Sngl    61140    61996         +         0

 >2351068        /28601

    len =     2200    nex =        7

      Init    65723    65950         +         0
      Intr    66035    66198         +         0
      Intr    66298    66349         +         0
      Intr    66544    66771         +         0
      Intr    66874    67063         +         0
      Intr    67153    67418         +         0
      Term    67680    67922         +         0

 >2351068        /25211

    len =     1220    nex =        1

      Sngl    69965    68746         -         0

 >2351069        /26016

    len =     2002    nex =        7

      Init    26231    26670         +         0
      Intr    26762    26870         +         0
      Intr    26960    27122         +         0
      Intr    27209    27357         +         0
      Intr    27450    27601         +         0
      Intr    27686    27800         +         0
      Term    27886    28232         +         0

 >2351069        /1271

    len =     3480    nex =        9

      Init    42775    42864         +         0
      Intr    43235    43369         +         0
      Intr    43517    43633         +         0
      Intr    43791    43942         +         0
      Intr    44014    44098         +         0
      Intr    44277    44371         +         0
      Intr    44852    45017         +         0
```

```
     Intr   45150   45345        +      0
     Term   45434   45819        +      0

 >2351069       /13271

    len =     702   nex =      3

     Init   62856   62885        +      0
     Intr   62964   63042        +      0
     Term   63127   63557        +      0

 >2351069       /7744

    len =    1618   nex =      8

     Term   67305   66948        -      0
     Intr   67508   67411        -      0
     Intr   67723   67598        -      0
     Intr   67896   67813        -      0
     Intr   68098   67982        -      0
     Intr   68261   68178        -      0
     Intr   68427   68380        -      0
     Init   68562   68508        -      0

 >2351069       /3285

    len =    3163   nex =     13

     Term   67262   67061        -      0
     Intr   67508   67411        -      0
     Intr   67723   67598        -      0
     Intr   67896   67813        -      0
     Intr   68098   67982        -      0
     Intr   68261   68178        -      0
     Intr   68427   68380        -      0
     Intr   68562   68508        -      0
     Intr   68759   68704        -      0
     Intr   68928   68844        -      0
     Intr   69102   69029        -      0
     Intr   69415   69349        -      0
     Init   70098   70008        -      0

 >2351070       /97197

    len =     697   nex =      1

     Sngl   23957   23261        -      0

 >2351070       /6363

    len =     560   nex =      1

     Sngl   34956   34397        -      0

 >2351070       /26053

    len =     817   nex =      1

     Sngl   46123   46936        +      0
```

```
>2351071        /17432

   len =     2313    nex =        9

    Term   46885    46586         -        0
    Intr   47174    47088         -        0
    Intr   47356    47291         -        0
    Intr   47556    47467         -        0
    Intr   47720    47640         -        0
    Intr   47910    47833         -        0
    Intr   48093    48003         -        0
    Intr   48436    48295         -        0
    Init   48898    48628         -        0

>2351071        /39195

   len =     2186    nex =        3

    Term   70730    70227         -        0
    Intr   71606    71158         -        0
    Init   72412    72145         -        0

>2351071        /17360

   len =     1402    nex =        3

    Term   78193    77927         -        0
    Intr   78535    78274         -        0
    Init   79311    79168         -        0

>2351071        /26743

   len =     1466    nex =        3

    Term   78193    77927         -        0
    Intr   78535    78274         -        0
    Init   79392    79168         -        0

>2351072        /29659

   len =     2508    nex =        5

    Term   22869    22279         -        0
    Intr   23128    23019         -        0
    Intr   23667    23238         -        0
    Intr   23978    23838         -        0
    Init   24786    24671         -        0

>2351072        /207148

   len =      797    nex =        1

    Sngl   50991    50195         -        0

>2351073        /98326

   len =      676    nex =        3

    Term   19588    19334         -        0
    Intr   19757    19681         -        0
```

```
     Init    19996   19838          -        0

>2351073          /100141

   len =      1717    nex =         5

     Term    19588   19293          -        0
     Intr    19757   19681          -        0
     Intr    20220   19838          -        0
     Intr    20633   20533          -        0
     Init    21009   20902          -        0

>2351073          /115914

   len =       116    nex =         1

     Sngl    26710   26595          -        0

>2351073          /95599

   len =       749    nex =         3

     Term    26967   26608          -        0
     Intr    27178   27047          -        0
     Init    27356   27258          -        0

>2351073          /35552

   len =      1828    nex =         6

     Term    26967   26653          -        0
     Intr    27178   27047          -        0
     Intr    27399   27258          -        0
     Intr    27742   27550          -        0
     Intr    28087   27842          -        0
     Init    28480   28170          -        0

>2351073          /118777

   len =      1030    nex =         1

     Sngl    31871   32900          +        0

>2358139          /20380

   len =       876    nex =         3

     Init    15794   15936          +        0
     Intr    16035   16176          +        0
     Term    16428   16669          +        0

>2358139          /29808

   len =      1270    nex =         2

     Term    64249   63873          -        0
     Init    65100   64760          -        0

>2358139          /108558
```

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```
   len =    1069   nex =       3

    Init   65271   65413         +        0
    Intr   65781   65860         +        0
    Term   66116   66339         +        0

>2358139        /1730

   len =    1484   nex =       3

    Init   71725   71848         +        0
    Intr   72291   72590         +        0
    Term   72701   73208         +        0

>2392762        /8805

   len =    1259   nex =       2

    Term   30586   29909         -        0
    Init   31167   30868         -        0

>2392762        /14724

   len =    1796   nex =       8

    Term   60877   60621         -        0
    Intr   61051   60973         -        0
    Intr   61293   61140         -        0
    Intr   61514   61420         -        0
    Intr   61620   61585         -        0
    Intr   61952   61727         -        0
    Intr   62107   62037         -        0
    Init   62416   62342         -        0

>2392762        /15990

   len =    1729   nex =       8

    Term   60877   60688         -        0
    Intr   61051   60973         -        0
    Intr   61293   61140         -        0
    Intr   61514   61420         -        0
    Intr   61620   61585         -        0
    Intr   61952   61727         -        0
    Intr   62107   62037         -        0
    Init   62416   62342         -        0

>2392762        /41162

   len =     951   nex =       3

    Init   68249   68350         +        0
    Intr   68449   68513         +        0
    Term   68901   69199         +        0

>2435510        /32833

   len =    1450   nex =       5

    Term   41015   40654         -        0
   Intr   41265   41098         -        0
   Intr   41451   41368         -        0
   Intr   41718   41540         -        0
   Init   42097   41892         -        0

>2435510        /1011

  len =     1120   nex =       3

   Term   51801   51490         -        0
   Intr   52028   51949         -        0
   Init   52609   52122         -        0

>2435510        /19362

  len =     1041   nex =       3

   Init   61031   61254         +        0
   Intr   61359   61535         +        0
   Term   61610   62071         +        0

>2435510        /142314

  len =      919   nex =       3

   Init   61151   61254         +        0
   Intr   61359   61535         +        0
   Term   61610   62069         +        0

>2435510        /33456

  len =     2142   nex =       6

   Term    4364    4051         -        0
   Intr    4676    4612         -        0
   Intr    5214    5151         -        0
   Intr    5423    5314         -        0
   Intr    5600    5513         -        0
   Init    6192    5794         -        0

>2435510        /4367

  len =     2039   nex =       8

   Init   76018   76119         +        0
   Intr   76377   76574         +        0
   Intr   76648   76707         +        0
   Intr   76793   77235         +        0
   Intr   77335   77501         +        0
   Intr   77587   77660         +        0
   Intr   77749   77808         +        0
   Term   77912   78053         +        0

>2443899        /22008

  len =     1489   nex =       2

   Term  102074  101797         -        0
   Init  103282  102296         -        0
```

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```
>2443899        /1734

   len =      888    nex =        2

    Term   14747    14318          -        0
    Init   15205    14965          -        0

>2459406        /42992

   len =     2396    nex =       10

    Term  117911   117825          -        0
    Intr  118071   117986          -        0
    Intr  118340   118166          -        0
    Intr  118518   118458          -        0
    Intr  118661   118595          -        0
    Intr  118838   118754          -        0
    Intr  119077   118920          -        0
    Intr  119310   119166          -        0
    Intr  119486   119427          -        0
    Init  119855   119575          -        0

>2459406        /11254

   len =     2035    nex =        6

    Init  128392   128598          +        0
    Intr  128894   129063          +        0
    Intr  129142   129327          +        0
    Intr  129412   129577          +        0
    Intr  129681   129870          +        0
    Term  130089   130426          +        0

>2459406        /92741

   len =      538    nex =        1

    Sngl  141230   140693          -        0

>2459406        /13741

   len =     1713    nex =        4

    Term   18475    18146          -        0
    Intr   18628    18567          -        0
    Intr   19123    18713          -        0
    Init   19858    19394          -        0

>2459406        /25272

   len =     1750    nex =        4

    Init    2679     2985          +        0
    Intr    3377     3419          +        0
    Intr    3511     3571          +        0
    Term    3697     4419          +        0

>2459406        /35273

   len =     2218    nex =        3
```

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```
     Term   26889   26777        -       0
     Intr   28208   27837        -       0
     Init   28994   28459        -       0

>2459406        /28563

    len =     1150   nex =       4

     Term   47656   47428        -       0
     Intr   47792   47751        -       0
     Intr   48158   47874        -       0
     Init   48577   48488        -       0

>2459406        /119409

    len =      468   nex =       1

     Sngl   57470   57023        -       0

>2459406        /116034

    len =      337   nex =       1

     Sngl   61222   61558        +       0

>2459406        /8717

    len =     2113   nex =       4

     Init   66546   66940        +       0
     Intr   67084   67181        +       0
     Intr   67274   67339        +       0
     Term   68443   68658        +       0

>2459406        /31633

    len =      945   nex =       2

     Init   77435   77674        +       0
     Term   78004   78379        +       0

>2459406        /19302

    len =     2115   nex =       6

     Term   80490   80306        -       0
     Intr   80717   80586        -       0
     Intr   80949   80814        -       0
     Intr   81174   81044        -       0
     Intr   81479   81424        -       0
     Init   82420   82270        -       0

>2459406        /37919

    len =     2274   nex =       6

     Term   80490   80262        -       0
     Intr   80717   80586        -       0
     Intr   80949   80814        -       0
```

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```
      Intr   81174   81044         -        0
      Intr   81479   81424         -        0
      Init   82535   82270         -        0

 >2459406        /18894

     len =      235    nex =        1

      Sngl   85070   85304         +        0

 >2477521        /15308

     len =     1434    nex =        1

      Sngl   11192   12625         +        0

 >2477521        /27205

     len =      760    nex =        3

      Term   22663   22447         -        0
      Intr   22864   22743         -        0
      Init   23206   22955         -        0

 >2477521        /40049

     len =     3210    nex =        5

      Init   52491   52536         +        0
      Intr   52618   52732         +        0
      Intr   52824   52891         +        0
      Intr   52986   53708         +        0
      Term   53792   54336         +        0

 >2477521        /3549

     len =     1750    nex =        4

      Init   59783   60056         +        0
      Intr   60329   60677         +        0
      Intr   60773   60914         +        0
      Term   60979   61527         +        0

 >2477521        /12293

     len =     1796    nex =        6

      Term   71123   70636         -        0
      Intr   71380   71205         -        0
      Intr   71502   71478         -        0
      Intr   71702   71620         -        0
      Intr   72024   71951         -        0
      Init   72431   72108         -        0

 >2477521        /98850

     len =     4463    nex =        7

      Init   74583   74814         +        0
      Intr   77407   77441         +        0
```

```
    Intr   77553   77614         +       0
    Intr   77696   77795         +       0
    Intr   77904   77945         +       0
    Intr   78281   78322         +       0
    Term   78695   79045         +       0

>2477521       /92459

   len =    4460   nex =       7

    Init   74588   74814         +       0
    Intr   77285   77342         +       0
    Intr   77553   77614         +       0
    Intr   77696   77795         +       0
    Intr   77904   77945         +       0
    Intr   78281   78322         +       0
    Term   78695   79047         +       0

>2477521       /5076

   len =     730   nex =       3

    Term   79591   79372         -       0
    Intr   79924   79697         -       0
    Init   80096   80042         -       0

>2477521       /4033

   len =    1930   nex =       7

    Init   94403   94493         +       0
    Intr   94625   94761         +       0
    Intr   94865   94911         +       0
    Intr   94999   95483         +       0
    Intr   95570   95727         +       0
    Intr   95814   95975         +       0
    Term   96051   96327         +       0

>2494106       /36412

   len =    1375   nex =       3

    Term   99606   98923         -       0
    Intr  100124   99692         -       0
    Init  100297  100214         -       0

>2494106       /11408

   len =     644   nex =       1

    Sngl  109531  110174         +       0

>2494106       /8951

   len =     910   nex =       1

    Sngl  112974  112773         -       0

>2494106       /37020
```

```
   len =      757    nex =        4

    Term  122980   122712          -         0
    Intr  123133   123078          -         0
    Intr  123278   123220          -         0
    Init  123468   123370          -         0

>2494106        /29872

   len =      861    nex =        2

    Term  122980   122712          -         0
    Init  123133   123078          -         0

>2494106        /34434

   len =      866    nex =        4

    Term  122980   122714          -         0
    Intr  123133   123078          -         0
    Intr  123278   123220          -         0
    Init  123577   123370          -         0

>2494106        /34374

   len =      359    nex =        2

    Term  123278   123219          -         0
    Init  123577   123370          -         0

>2494106        /5465

   len =     2050    nex =        7

    Init  132597   132734          +         0
    Intr  133129   133207          +         0
    Intr  133336   133389          +         0
    Intr  133680   133793          +         0
    Intr  134040   134107          +         0
    Intr  134190   134301          +         0
    Term  134381   134640          +         0

>2494106        /1520

   len =     1810    nex =        6

    Init  132677   133207          +         0
    Intr  133336   133389          +         0
    Intr  133680   133793          +         0
    Intr  134040   134107          +         0
    Intr  134190   134301          +         0
    Term  134381   134477          +         0

>2494106        /2681

   len =      910    nex =        1

    Sngl  143514   143911          +         0

>2494106        /33770
```

```
     len =    1302   nex =       4

      Term  158712  158351          -         0
      Intr  159059  158976          -         0
      Intr  159236  159156          -         0
      Init  159509  159332          -         0

  >2494106        /27457

     len =    1462   nex =       4

      Term   40898   40547          -         0
      Intr   41137   41003          -         0
      Intr   41443   41231          -         0
      Init   42008   41526          -         0

  >2494106        /25255

     len =    1719   nex =       3

      Init   54004   54063          +         0
      Intr   54151   54486          +         0
      Term   54639   54877          +         0

  >2494106        /14939

     len =     610   nex =       2

      Init   54277   54486          +         0
      Term   54639   54879          +         0

  >2494106        /32130

     len =    3130   nex =      12

      Term   56042   55686          -         0
      Intr   56181   56114          -         0
      Intr   56328   56265          -         0
      Intr   56502   56421          -         0
      Intr   56676   56618          -         0
      Intr   56984   56925          -         0
      Intr   57266   57104          -         0
      Intr   57498   57374          -         0
      Intr   57857   57795          -         0
      Intr   58060   58001          -         0
      Intr   58325   58140          -         0
      Init   58811   58689          -         0

  >2494106        /6667

     len =    1554   nex =       1

      Sngl   60644   59091          -         0

  >2494106        /25894

     len =    1630   nex =       4

      Term   64139   63599          -         0
```

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```
         Intr    64439    64381          -          0
         Intr    64965    64855          -          0
         Init    65226    65138          -          0

   >2494110         /23300

        len =     2036    nex =         5

         Term    17775    17469          -          0
         Intr    18041    17877          -          0
         Intr    18302    18159          -          0
         Intr    18618    18423          -          0
         Init    19504    19053          -          0

   >2494110         /8559

        len =     1302    nex =         2

         Init    25200    25402          +          0
         Term    26210    26501          +          0

   >2494110         /37952

        len =     4214    nex =         6

         Init    25200    25402          +          0
         Intr    26210    26290          +          0
         Intr    27617    28259          +          0
         Intr    28358    28461          +          0
         Intr    28571    28709          +          0
         Term    28803    29413          +          0

   >2494110         /21100

        len =      812    nex =         3

         Term    30699    30410          -          0
         Intr    30921    30796          -          0
         Init    31221    30993          -          0

   >2494110         /34753

        len =      807    nex =         3

         Term    30699    30415          -          0
         Intr    30921    30796          -          0
         Init    31221    30993          -          0

   >2494110         /110726

        len =      493    nex =         1

         Sngl    32194    32672          +          0

   >2494110         /2265

        len =      494    nex =         1

         Sngl    38819    39312          +          0
```

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```
>2494110        /13232

   len =     1220    nex =        1

     Sngl   40544   39752          -         0

>2494110        /31923

   len =     1284    nex =        3

     Init   41985   42310          +         0
     Intr   42859   42930          +         0
     Term   43017   43268          +         0

>2494110        /100984

   len =     1340No match - No prediction
>2494110        /27110

   len =      108    nex =        1

     Sngl   74373   74480          +         0

>2494110        /40608

   len =     1703    nex =        5

     Term   91321   90966          -         0
     Intr   91466   91405          -         0
     Intr   91657   91540          -         0
     Intr   92025   91739          -         0
     Init   92668   92298          -         0

>2494110        /2935

   len =     1613    nex =        5

     Init   97175   97627          +         0
     Intr   97725   97897          +         0
     Intr   97974   98088          +         0
     Intr   98324   98478          +         0
     Term   98578   98787          +         0

>2505864        /35333

   len =     1426    nex =        4

     Init   20951   21020          +         0
     Intr   21255   21415          +         0
     Intr   21681   21869          +         0
     Term   22136   22367          +         0

>2505864        /4328

   len =     1374    nex =        4

     Init   21013   21070          +         0
     Intr   21255   21415          +         0
     Intr   21681   21869          +         0
     Term   22136   22386          +         0
```

```
>2505873        /6115

   len =     1600    nex =        2

     Term   14088    13696         -         0
     Init   15295    14419         -         0

>2505873        /33852

   len =      134    nex =        1

     Sngl   19922    20055         +         0

>2505873        /36699

   len =      236    nex =        1

     Sngl   27483    27718         +         0

>2529657        /32457

   len =     1232    nex =        5

     Term   10325    10206         -         0
     Intr   10512    10408         -         0
     Intr   10777    10703         -         0
     Intr   11135    11111         -         0
     Init   11437    11243         -         0

>2529657        /26123

   len =     1422    nex =        5

     Term   10325    10041         -         0
     Intr   10512    10408         -         0
     Intr   10777    10703         -         0
     Intr   11135    11111         -         0
     Init   11462    11380         -         0

>2529657        /20647

   len =     1390    nex =        5

     Term   12109    11630         -         0
     Intr   12283    12185         -         0
     Intr   12499    12362         -         0
     Intr   12722    12592         -         0
     Init   13015    12840         -         0

>2529657        /28691

   len =     2057    nex =        7

     Term   12109    11913         -         0
     Intr   12283    12185         -         0
     Intr   12499    12362         -         0
     Intr   12722    12592         -         0
     Intr   12986    12840         -         0
     Intr   13647    13615         -         0
```

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```
        Init    13969    13735        -         0

    >2529657          /33373

        len =     2492    nex =        8

        Term    12109    11637        -         0
        Intr    12283    12185        -         0
        Intr    12499    12362        -         0
        Intr    12722    12592        -         0
        Intr    12986    12840        -         0
        Intr    13647    13615        -         0
        Intr    13831    13735        -         0
        Init    14128    13974        -         0

    >2529657          /24272

        len =     1054    nex =        4

        Term    17370    17243        -         0
        Intr    17555    17463        -         0
        Intr    17935    17637        -         0
        Init    18296    18094        -         0

    >2529657          /6394

        len =     1870    nex =        5

        Term    17370    16988        -         0
        Intr    17555    17463        -         0
        Intr    17935    17637        -         0
        Intr    18295    18094        -         0
        Init    18459    18415        -         0

    >2529657          /25729

        len =      802    nex =        1

        Sngl     3834     4635        +         0

    >2529657          /37870

        len =     3805    nex =       17

        Term    46706    46424        -         0
        Intr    46947    46882        -         0
        Intr    47087    47058        -         0
        Intr    47280    47182        -         0
        Intr    47466    47371        -         0
        Intr    47623    47573        -         0
        Intr    47773    47707        -         0
        Intr    47950    47856        -         0
        Intr    48158    48077        -         0
        Intr    48324    48275        -         0
        Intr    48463    48413        -         0
        Intr    48638    48540        -         0
        Intr    49052    48969        -         0
        Intr    49302    49192        -         0
        Intr    49575    49426        -         0
        Intr    49795    49678        -         0
```

```
     Init   50050   49884       -      0

>2529657        /32039

    len =     670    nex =      1

     Sngl   63987   64654       +      0

>2529657        /9499

    len =     654    nex =      2

     Term   65131   64658       -      0
     Init   65297   65222       -      0

>2529657        /38461

    len =    2350    nex =     10

     Term   65131   64823       -      0
     Intr   65346   65222       -      0
     Intr   65588   65432       -      0
     Intr   65777   65686       -      0
     Intr   65890   65863       -      0
     Intr   66093   65976       -      0
     Intr   66394   66339       -      0
     Intr   66604   66507       -      0
     Intr   66777   66693       -      0
     Init   67165   66986       -      0

>2529657        /13774

    len =    2397    nex =     10

     Term   65131   64823       -      0
     Intr   65346   65222       -      0
     Intr   65588   65432       -      0
     Intr   65777   65686       -      0
     Intr   65890   65863       -      0
     Intr   66093   65976       -      0
     Intr   66394   66339       -      0
     Intr   66604   66507       -      0
     Intr   66777   66693       -      0
     Init   67219   66986       -      0

>2529657        /34914

    len =     717    nex =      1

     Sngl   75255   74539       -      0

>2529657        /37980

    len =    1352    nex =      1

     Sngl   75893   74542       -      0

>2564044        /156017

    len =     401    nex =      1
```

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```
    Sngl   12975   12575        -       0

 >2564044       /156773

    len =     350    nex =      1

    Sngl   12997   12648        -       0

 >2564044       /31129

    len =     430    nex =      1

    Sngl   13041   12616        -       0

 >2564044       /21629

    len =    1610    nex =      5

    Term   36986   36739        -       0
    Intr   37123   37068        -       0
    Intr   37318   37272        -       0
    Intr   37669   37626        -       0
    Init   38348   38232        -       0

 >2564044       /22860

    len =    3400    nex =     11

    Init    5043    5315        +       0
    Intr    5670    5734        +       0
    Intr    5871    5969        +       0
    Intr    6171    6303        +       0
    Intr    6748    6807        +       0
    Intr    6897    7019        +       0
    Intr    7379    7450        +       0
    Intr    7562    7699        +       0
    Intr    7786    7941        +       0
    Intr    8028    8132        +       0
    Term    8282    8442        +       0

 >2564045       /108335

    len =    1516    nex =      2

    Term     653     118        -       0
    Init    1633     770        -       0

 >2564045       /512

    len =    1435    nex =      1

    Sngl   40196   39668        -       0

 >2564045       /40250

    len =    1210    nex =      2

    Term   57008   56234        -       0
    Init   57441   57096        -       0
```

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```
>2564045       /36090

   len =    1219   nex =       2

    Term   57008   56234       -        0
    Init   57452   57096       -        0

>2564045       /33763

   len =    1217   nex =       1

    Sngl    5886    7102       +        0

>2564045       /23566

   len =    1043   nex =       3

    Init    9042    9192       +        0
    Intr    9618    9763       +        0
    Term    9851   10084       +        0

>2564046       /4272

   len =    4185   nex =      11

    Term   18249   17894       -        0
    Intr   18506   18454       -        0
    Intr   18683   18598       -        0
    Intr   18985   18867       -        0
    Intr   19502   19431       -        0
    Intr   19881   19708       -        0
    Intr   20444   20289       -        0
    Intr   20917   20836       -        0
    Intr   21276   21130       -        0
    Intr   21654   21468       -        0
    Init   22078   21842       -        0

>2564046       /13993

   len =    1672   nex =       6

    Init   27089   27339       +        0
    Intr   27573   27725       +        0
    Intr   27820   27972       +        0
    Intr   28179   28262       +        0
    Intr   28344   28485       +        0
    Term   28581   28760       +        0

>2564046       /35683

   len =     697   nex =       3

    Term   34417   34208       -        0
    Intr   34609   34504       -        0
    Init   34904   34742       -        0

>2564047       /12802

   len =    1648   nex =       3
```

```
    Init   16402   16518        +        0
    Intr   17081   17129        +        0
    Term   17663   17714        +        0

>2564047        /19442

   len =     850   nex =        1

    Sngl   21861   22701        +        0

>2564047        /2533

   len =    1779   nex =        3

    Init   37480   37886        +        0
    Intr   37970   38637        +        0
    Term   39199   39258        +        0

>2564047        /32890

   len =    1279   nex =        1

    Sngl   51389   50111        -        0

>2564047        /13737

   len =    1302   nex =        5

    Term   57880   57668        -        0
    Intr   58070   58011        -        0
    Intr   58297   58197        -        0
    Intr   58633   58398        -        0
    Init   58969   58725        -        0

>2564047        /6893

   len =    1309   nex =        5

    Term   57880   57662        -        0
    Intr   58070   58011        -        0
    Intr   58297   58197        -        0
    Intr   58633   58398        -        0
    Init   58970   58725        -        0

>2564047        /114864

   len =    2470   nex =        9

    Init   59318   59464        +        0
    Intr   59652   59723        +        0
    Intr   59821   59895        +        0
    Intr   60508   60588        +        0
    Intr   60854   60923        +        0
    Intr   60996   61087        +        0
    Intr   61178   61219        +        0
    Intr   61298   61378        +        0
    Term   61566   61785        +        0

>2564047        /105566
```

```
                                    979

    len =     979    nex =        1

      Sngl   62070   63048         +        0

>2564047        /12455

    len =    1933    nex =        2

      Term   67046   66415         -        0
      Init   68347   67916         -        0

>2564047        /40711

    len =     850    nex =        1

      Sngl   78369   77529         -        0

>2564048        /105906

    len =    1212    nex =        2

      Init    2380    2769         +        0
      Term    2946    3591         +        0

>2564048        /115613

    len =     586    nex =        1

      Sngl   31514   30929         -        0

>2564048        /1200

    len =    1778    nex =        4

      Term   38609   37885         -        0
      Intr   38864   38681         -        0
      Intr   39244   38988         -        0
      Init   39662   39331         -        0

>2564048        /39462

    len =    2351    nex =        7

      Init   41518   41825         +        0
      Intr   42059   42268         +        0
      Intr   42387   42557         +        0
      Intr   42766   42889         +        0
      Intr   43155   43216         +        0
      Intr   43305   43386         +        0
      Term   43481   43868         +        0

>2564048        /10292

    len =    1951    nex =        7

      Init   41667   41825         +        0
      Intr   42059   42268         +        0
      Intr   42387   42557         +        0
      Intr   42766   42889         +        0
```

```
     Intr    43155    43216        +        0
     Intr    43305    43386        +        0
     Term    43481    43617        +        0

>2564048        /26637

    len =     1980    nex =        7

     Init    61938    62027        +        0
     Intr    62306    62497        +        0
     Intr    62586    62757        +        0
     Intr    62859    62932        +        0
     Intr    63011    63037        +        0
     Intr    63126    63149        +        0
     Term    63245    63657        +        0

>2564048        /158431

    len =     1690    nex =        5

     Init    65258    65519        +        0
     Intr    65699    65751        +        0
     Intr    65845    65980        +        0
     Intr    66115    66290        +        0
     Term    66365    66942        +        0

>2564049        /37294

    len =     1427    nex =        2

     Term      485      294        -        0
     Init     1720      625        -        0

>2564049        /104793

    len =     1873    nex =        7

     Init    17973    18128        +        0
     Intr    18663    18789        +        0
     Intr    18882    19035        +        0
     Intr    19112    19208        +        0
     Intr    19304    19392        +        0
     Intr    19521    19589        +        0
     Term    19790    19845        +        0

>2564049        /141731

    len =     2068    nex =        7

     Init    18007    18128        +        0
     Intr    18663    18789        +        0
     Intr    18882    19035        +        0
     Intr    19112    19208        +        0
     Intr    19304    19392        +        0
     Intr    19521    19589        +        0
     Term    19790    20074        +        0

>2564049        /21604

    len =      557    nex =        1
```

```
  Sngl   28618   28062         -        0

>2564049       /16144

 len =    1365   nex =       3

  Init   28919   29348         +        0
  Intr   29603   29695         +        0
  Term   30029   30283         +        0

>2564049       /31971

 len =    1818   nex =       2

  Init   35677   36089         +        0
  Term   36890   37494         +        0

>2564049       /13667

 len =    1704   nex =       6

  Term    5026    4812         -        0
  Intr    5207    5118         -        0
  Intr    5466    5299         -        0
  Intr    5691    5572         -        0
  Intr    5932    5787         -        0
  Init    6515    6354         -        0

>2564050        /6203

 len =    2839   nex =      13

  Init   12017   12391         +        0
  Intr   12485   12567         +        0
  Intr   12820   12974         +        0
  Intr   13048   13082         +        0
  Intr   13144   13293         +        0
  Intr   13467   13562         +        0
  Intr   13634   13750         +        0
  Intr   13832   13951         +        0
  Intr   14029   14121         +        0
  Intr   14202   14324         +        0
  Intr   14407   14523         +        0
  Intr   14606   14668         +        0
  Term   14766   14842         +        0

>2564050       /123496

 len =     674   nex =       1

  Sngl   17696   18369         +        0

>2564050       /16313

 len =    1594   nex =       5

  Init    2671    2918         +        0
  Intr    3227    3325         +        0
  Intr    3410    3518         +        0
```

```
     Intr     3687    3758          +         0
     Term     3993    4264          +         0

>2564050        /14738

    len =     1040   nex =        2

     Term    28103   27853          -         0
     Init    28892   28606          -         0

>2564050        /13951

    len =     1063   nex =        2

     Term    28103   27848          -         0
     Init    28910   28606          -         0

>2564050        /38057

    len =     2006   nex =        0

>2564051        /7688

    len =     1722   nex =        2

     Term    13311   12928          -         0
     Init    13996   13887          -         0

>2564051        /6220

    len =     2570   nex =        6

     Init    18254   18493          +         0
     Intr    18575   18754          +         0
     Intr    19785   19904          +         0
     Intr    19917   20078          +         0
     Intr    20178   20459          +         0
     Term    20546   20823          +         0

>2564051        /30648

    len =     2334   nex =        9

     Init    33401   33589          +         0
     Intr    33676   33848          +         0
     Intr    34149   34268          +         0
     Intr    34373   34429          +         0
     Intr    34595   34675          +         0
     Intr    34763   34797          +         0
     Intr    34933   35006          +         0
     Intr    35103   35262          +         0
     Term    35380   35734          +         0

>2564051        /30994

    len =     2530   nex =        8

     Init    45513   45608          +         0
     Intr    46036   46115          +         0
     Intr    46206   46280          +         0
```

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```
     Intr   46370   46473         +       0
     Intr   46561   46717         +       0
     Intr   46810   46897         +       0
     Intr   46997   47069         +       0
     Term   47147   47224         +       0

>2564051       /29619

    len =     942   nex =       3

     Init   46810   46897         +       0
     Intr   46997   47069         +       0
     Term   47147   47224         +       0

>2564051       /29829

    len =    1317   nex =       3

     Term   48114   47710         -       0
     Intr   48493   48207         -       0
     Init   49026   48809         -       0

>2564051       /6519

    len =    1128   nex =       2

     Init   72721   72978         +       0
     Term   73194   73848         +       0

>2564051       /142033

    len =     651   nex =       2

     Init   72788   72978         +       0
     Term   73194   73438         +       0

>2564051       /14159

    len =    1394   nex =       5

     Term   74311   74056         -       0
     Intr   74603   74398         -       0
     Intr   74863   74713         -       0
     Intr   75172   74950         -       0
     Init   75449   75412         -       0

>2564051       /40866

    len =    1519   nex =       5

     Term   74311   74064         -       0
     Intr   74603   74398         -       0
     Intr   74863   74713         -       0
     Intr   75172   74950         -       0
     Init   75582   75412         -       0

>2564051       /17770

    len =    1500   nex =       5
```

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```
      Term   74311   74086         -        0
      Intr   74603   74398         -        0
      Intr   74863   74713         -        0
      Intr   75172   74950         -        0
      Init   75445   75412         -        0

>2564051        /13949

    len =     1110   nex =        3

      Term   82879   82476         -        0
      Intr   83240   82973         -        0
      Init   83585   83325         -        0

>2570223        /40832

    len =     2253   nex =        6

      Init   17162   17477         +        0
      Intr   17799   17892         +        0
      Intr   18430   18609         +        0
      Intr   18688   18807         +        0
      Intr   18887   19020         +        0
      Term   19185   19414         +        0

>2570223        /37699

    len =     2869   nex =        9

      Term   26477   25979         -        0
      Intr   26840   26580         -        0
      Intr   27159   26941         -        0
      Intr   27498   27271         -        0
      Intr   27878   27776         -        0
      Intr   28077   27965         -        0
      Intr   28258   28197         -        0
      Intr   28478   28346         -        0
      Init   28847   28757         -        0

>2570223        /23106

    len =      629   nex =        1

      Sngl   74691   75319         +        0

>2583106        /29207

    len =     2272   nex =        5

      Init  108141  108430         +        0
      Intr  108875  109071         +        0
      Intr  109540  109629         +        0
      Intr  109744  109815         +        0
      Term  110152  110412         +        0

>2583106        /36389

    len =      643   nex =        1

      Sngl  121587  120945         -        0
```

```
>2583106        /17187

   len =     677    nex =        1

     Sngl  121618  120942          -          0

>2583106        /23203

   len =     704    nex =        2

     Init   13956   14106          +          0
     Term   14207   14659          +          0

>2583106        /2322

   len =     531    nex =        1

     Sngl   15480   14950          -          0

>2583106        /26817

   len =    1955    nex =        3

     Term   15998   14950          -          0
     Intr   16202   16119          -          0
     Init   16904   16559          -          0

>2583106        /7709

   len =    1471    nex =        1

     Sngl    3827    5297          +          0

>2583106        /33864

   len =    1700    nex =        1

     Sngl   64128   64474          +          0

>2583106        /27799

   len =    1690    nex =        4

     Term   73308   72358          -          0
     Intr   73553   73400          -          0
     Intr   73796   73648          -          0
     Init   74047   73886          -          0

>2583106        /15659

   len =    2848    nex =        7

     Term   73308   72358          -          0
     Intr   73553   73400          -          0
     Intr   73796   73648          -          0
     Intr   74168   73886          -          0
     Intr   74356   74251          -          0
     Intr   74536   74446          -          0
     Init   75205   74719          -          0
```

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```
>2583106       /18320

   len =    2568   nex =       7

    Term   73308   72638        -       0
    Intr   73553   73400        -       0
    Intr   73796   73648        -       0
    Intr   74168   73886        -       0
    Intr   74356   74251        -       0
    Intr   74536   74446        -       0
    Init   75205   74719        -       0

>2583106       /21765

   len =    1874   nex =       0

>2583106       /1969

   len =    5689   nex =       1

    Sngl   88355   88684        +       0

>2583106       /37127

   len =     310   nex =       0

>2583106       /37621

   len =    3101   nex =      15

    Term   89198   88862        -       0
    Intr   89371   89306        -       0
    Intr   89531   89462        -       0
    Intr   89689   89616        -       0
    Intr   89891   89793        -       0
    Intr   90037   89976        -       0
    Intr   90178   90137        -       0
    Intr   90316   90265        -       0
    Intr   90541   90442        -       0
    Intr   90682   90638        -       0
    Intr   90843   90796        -       0
    Intr   91179   91104        -       0
    Intr   91456   91286        -       0
    Intr   91590   91540        -       0
    Init   91962   91806        -       0

>2584827       /273

   len =    2260   nex =       5

    Term  101872  101586        -       0
    Intr  102093  102017        -       0
    Intr  102388  102242        -       0
    Intr  102650  102480        -       0
    Init  103150  102928        -       0

>2584827       /5480

   len =    1994   nex =       3
```

```
    Term  111925  111310        -        0
    Intr  112167  112049        -        0
    Init  113303  112263        -        0

>2584827       /5171

   len =     319   nex =       1

    Sngl  115114  114796        -        0

>2584827       /17426

   len =     597   nex =       1

    Sngl  115422  114826        -        0

>2584827       /11593

   len =     562   nex =       1

    Sngl  115422  114861        -        0

>2584827       /25571

   len =     610   nex =       1

    Sngl  115430  114821        -        0

>2584827       /34348

   len =    1756   nex =       8

    Term  117147  116843        -        0
    Intr  117385  117233        -        0
    Intr  117590  117483        -        0
    Intr  117734  117687        -        0
    Intr  118025  117813        -        0
    Intr  118181  118117        -        0
    Intr  118386  118262        -        0
    Init  118595  118482        -        0

>2584827       /39107

   len =    2383   nex =       7

    Term  117147  116840        -        0
    Intr  117385  117233        -        0
    Intr  117590  117483        -        0
    Intr  117734  117687        -        0
    Intr  118025  117813        -        0
    Intr  118181  118117        -        0
    Init  118386  118262        -        0

>2584827       /5712

   len =     790   nex =       1

    Sngl   23900   24682        +        0
```

```
>2584827        /27675

   len =      745    nex =        1

    Sngl   23978   24722          +        0

>2584827        /116395

   len =      286    nex =        1

    Sngl   29868   29583          -        0

>2584827        /4503

   len =      471    nex =        1

    Sngl   30113   29649          -        0

>2584827        /22292

   len =      592    nex =        1

    Sngl   30233   29642          -        0

>2584827        /25064

   len =      683    nex =        2

    Term   30138   29660          -        0
    Init   30342   30322          -        0

>2584827        /25142

   len =      816    nex =        2

    Term   30138   29596          -        0
    Init   30411   30322          -        0

>2584827        /1994

   len =      835    nex =        2

    Term   30138   29584          -        0
    Init   30418   30322          -        0

>2584827        /4479

   len =      655    nex =        3

    Term   84398   84092          -        0
    Intr   84584   84486          -        0
    Init   84746   84674          -        0

>2584827        /31676

   len =      649    nex =        2

    Term   85483   85211          -        0
    Init   85859   85582          -        0
```

```
>2584827       /32472

   len =    1762   nex =       7

    Term   84398   84113         -        0
    Intr   84584   84486         -        0
    Intr   84807   84674         -        0
    Intr   84997   84910         -        0
    Intr   85301   85255         -        0
    Intr   85483   85417         -        0
    Init   85870   85582         -        0

>2584827       /8972

   len =    4044   nex =      12

    Init   95183   95243         +        0
    Intr   95429   95523         +        0
    Intr   95608   95720         +        0
    Intr   95804   95972         +        0
    Intr   96059   96098         +        0
    Intr   96231   96295         +        0
    Intr   96387   96500         +        0
    Intr   96601   96665         +        0
    Intr   96783   96939         +        0
    Intr   97037   97156         +        0
    Intr   97247   97335         +        0
    Term   97422   97750         +        0

>2584827       /17473

   len =    1881   nex =       9

    Init   95871   95972         +        0
    Intr   96059   96098         +        0
    Intr   96231   96295         +        0
    Intr   96387   96500         +        0
    Intr   96601   96665         +        0
    Intr   96783   96939         +        0
    Intr   97037   97156         +        0
    Intr   97247   97335         +        0
    Term   97422   97751         +        0

>2618599       /23293

   len =     776   nex =       1

    Sngl   11508   11343         -        0

>2618599       /6500

   len =     997   nex =       1

    Sngl   13043   14039         +        0

>2618599       /40212

   len =    1750   nex =       3

    Term   12611   12066         -        0
```

```
     Intr    13271   13086         -        0
     Init    13808   13354         -        0

>2618599        /39514

    len =     1757   nex =        4

     Term    23461   23198         -        0
     Intr    24300   24198         -        0
     Intr    24625   24565         -        0
     Init    24954   24707         -        0

>2618599        /8490

    len =     1570   nex =        5

     Term    27344   27150         -        0
     Intr    27528   27425         -        0
     Intr    27900   27615         -        0
     Intr    28171   27989         -        0
     Init    28711   28527         -        0

>2618599        /96

    len =     1631   nex =        5

     Term    27344   27085         -        0
     Intr    27528   27425         -        0
     Intr    27900   27615         -        0
     Intr    28171   27989         -        0
     Init    28715   28527         -        0

>2618599        /96124

    len =      490   nex =        1

     Sngl    61246   60764         -        0

>2618599        /13096

    len =     1229   nex =        3

     Init    71559   71780         +        0
     Intr    72273   72395         +        0
     Term    72494   72787         +        0

>2618599        /93205

    len =      977   nex =        3

     Init    71649   71780         +        0
     Intr    72273   72395         +        0
     Term    72494   72625         +        0

>2618599        /34629

    len =     1114   nex =        3

     Init    71649   71780         +        0
     Intr    72273   72395         +        0
```

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```
          Term    72494    72762           +        0

     >2618599          /29341

        len =     1153    nex =        3

          Init    71650    71780           +        0
          Intr    72273    72395           +        0
          Term    72494    72802           +        0

     >2618599          /40708

        len =     1885    nex =        5

          Term    73223    72970           -        0
          Intr    73417    73329           -        0
          Intr    74198    74135           -        0
          Intr    74521    74458           -        0
          Init    74712    74609           -        0

     >2618599          /37431

        len =     2030    nex =        9

          Term    77559    77489           -        0
          Intr    77742    77644           -        0
          Intr    78021    77830           -        0
          Intr    78386    78249           -        0
          Intr    78575    78489           -        0
          Intr    78745    78659           -        0
          Intr    79060    78899           -        0
          Intr    79293    79157           -        0
          Init    79506    79391           -        0

     >2618600          /33948

        len =      508    nex =        1

          Sngl    11449    11709           +        0

     >2618600          /5511

        len =     2590    nex =        6

          Term    24538    24373           -        0
          Intr    24781    24651           -        0
          Intr    24969    24879           -        0
          Intr    25370    25228           -        0
          Intr    25905    25465           -        0
          Init    26961    26323           -        0

     >2618600          /28326

        len =     1413    nex =        2

          Term    34043    33702           -        0
          Init    35114    34932           -        0

     >2618600          /25219
```

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```
    len =     1534    nex =        3

      Init    36892    37172         +        0
      Intr    37302    37699         +        0
      Term    37781    38425         +        0

>2618600        /16323

    len =      564    nex =        1

      Sngl    38835    39398         +        0

>2618600        /19433

    len =     3202    nex =        3

      Init    54780    54996         +        0
      Intr    55178    55275         +        0
      Term    55405    57379         +        0

>2618600        /17022

    len =     1656    nex =        0

>2618600        /34218

    len =     2898    nex =        6

      Init    70436    70933         +        0
      Intr    71361    71426         +        0
      Intr    71892    72011         +        0
      Intr    72098    72382         +        0
      Intr    72467    72739         +        0
      Term    72924    73333         +        0

>2618600        /40280

    len =     1001    nex =        3

      Term    80824    80546         -        0
      Intr    81167    81118         -        0
      Init    81369    81257         -        0

>2618601        /98459

    len =     1457    nex =        3

      Term    19035    18675         -        0
      Intr    19433    19407         -        0
      Init    20131    19678         -        0

>2618601        /38593

    len =     1416    nex =        3

      Term    19035    18762         -        0
      Intr    19433    19407         -        0
      Init    20177    19678         -        0

>2618601        /3459
```

```
     len =     1481    nex =        3

       Term    24639    24049          -         0
       Intr    24900    24741          -         0
       Init    25529    25043          -         0

>2618601         /41015

     len =       90    nex =        1

       Sngl     2878     2789          -         0

>2618601         /2577

     len =     1537    nex =        3

       Term    28396    27900          -         0
       Intr    28801    28642          -         0
       Init    29436    28936          -         0

>2618601         /23349

     len =     1680    nex =        3

       Term    33360    32729          -         0
       Intr    33823    33661          -         0
       Init    34408    33933          -         0

>2618601         /10032

     len =     1835    nex =        5

       Term    37993    37806          -         0
       Intr    38138    38078          -         0
       Intr    38275    38222          -         0
       Intr    38435    38388          -         0
       Init    38573    38511          -         0

>2618601         /28362

     len =      876    nex =        2

       Term    41130    40811          -         0
       Init    41686    41620          -         0

>2618601         /36501

     len =      970    nex =        2

       Term    41130    40731          -         0
       Init    41698    41620          -         0

>2618601         /31107

     len =     1531    nex =        4

       Init    44336    44550          +         0
       Intr    44852    44969          +         0
       Intr    45277    45424          +         0
```

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```
    Term   45511   45866        +       0

>2618602       /16835

   len =     1213   nex =       3

    Init     1864    2123       +       0
    Intr     2566    2722       +       0
    Term     2846    3076       +       0

>2618602       /30658

   len =     1570   nex =       5

    Term   22604   22192        -       0
    Intr   22912   22693        -       0
    Intr   23242   23001        -       0
    Intr   23527   23315        -       0
    Init   23761   23601        -       0

>2618602       /37357

   len =     1215   nex =       1

    Sngl   28713   29927        +       0

>2618602       /25423

   len =      677   nex =       1

    Sngl   46717   47393        +       0

>2618602       /99873

   len =      900   nex =       1

    Sngl   54255   53356        -       0

>2618602       /7672

   len =      267   nex =       1

    Sngl   59695   59434        -       0

>2618602       /10243

   len =     1424   nex =       5

    Term   59695   59432        -       0
    Intr   59897   59845        -       0
    Intr   60072   60011        -       0
    Intr   60287   60254        -       0
    Init   60855   60687        -       0

>2618602       /1888

   len =     1916   nex =       4

    Init   66033   66354        +       0
    Intr   66640   66827        +       0
```

```
     Intr   66906   67302          +        0
     Term   67408   67948          +        0

>2618602       /3109

    len =    4196   nex =       5

     Term   76502   75640          -        0
     Intr   76837   76585          -        0
     Intr   77507   76944          -        0
     Intr   78819   78722          -        0
     Init   79835   79282          -        0

>2618602       /43035

    len =    2160   nex =       8

     Term    6638    6237          -        0
     Intr    6992    6725          -        0
     Intr    7269    7075          -        0
     Intr    7586    7446          -        0
     Intr    7831    7765          -        0
     Intr    8009    7930          -        0
     Intr    8166    8088          -        0
     Init    8396    8321          -        0

>2618602       /36948

    len =    2038   nex =       8

     Term    6638    6378          -        0
     Intr    6992    6725          -        0
     Intr    7269    7075          -        0
     Intr    7586    7446          -        0
     Intr    7831    7765          -        0
     Intr    8009    7930          -        0
     Intr    8166    8088          -        0
     Init    8415    8321          -        0

>2618602       /22582

    len =    1527   nex =       5

     Term    7586    7446          -        0
     Intr    7831    7765          -        0
     Intr    8009    7930          -        0
     Intr    8166    8088          -        0
     Init    8415    8321          -        0

>2618603       /143299

    len =     678   nex =       1

     Sngl   13660   12983          -        0

>2618603       /17250

    len =    2810   nex =       7

     Term   22323   21890          -        0
    Intr   22694   22411        -        0
    Intr   22944   22785        -        0
    Intr   23379   23050        -        0
    Intr   23590   23464        -        0
    Intr   23755   23676        -        0
    Init   24699   24401        -        0

>2618603       /40850

  len =     2893   nex =       13

    Term   57315   57005        -        0
    Intr   57504   57416        -        0
    Intr   57696   57585        -        0
    Intr   57938   57801        -        0
    Intr   58165   58019        -        0
    Intr   58344   58270        -        0
    Intr   58476   58435        -        0
    Intr   58720   58601        -        0
    Intr   58890   58816        -        0
    Intr   59036   58974        -        0
    Intr   59195   59126        -        0
    Intr   59447   59288        -        0
    Init   59897   59584        -        0

>2618604       /17002

  len =     2138   nex =        7

    Term   48168   47893        -        0
    Intr   48328   48286        -        0
    Intr   48523   48450        -        0
    Intr   48712   48639        -        0
    Intr   49301   49226        -        0
    Intr   49476   49399        -        0
    Init   50030   49757        -        0

>2618604       /32773

  len =      419   nex =        1

    Sngl   50632   50223        -        0

>2618605       /34592

  len =     2890   nex =       10

    Term   16301   16104        -        0
    Intr   16453   16385        -        0
    Intr   16645   16540        -        0
    Intr   16810   16755        -        0
    Intr   17032   16973        -        0
    Intr   17226   17142        -        0
    Intr   17695   17601        -        0
    Intr   17967   17845        -        0
    Intr   18278   18215        -        0
    Init   18991   18733        -        0

>2618605       /15525
```

```
   len =     1379    nex =        3

     Init    19682    19907        +        0
     Intr    20580    20660        +        0
     Term    20864    21060        +        0

>2618605        /18394

   len =      924    nex =        0

>2618605        /37433

   len =     1210    nex =        2

     Init    19695    19907        +        0
     Term    20580    20660        +        0

>2618605        /98771

   len =     1392    nex =        3

     Init    19695    19907        +        0
     Intr    20580    20660        +        0
     Term    20864    21086        +        0

>2618605        /4869

   len =     1372    nex =        0

>2618605        /6170

   len =     1030    nex =        2

     Term    21605    21167        -        0
     Init    22188    21736        -        0

>2618605        /114037

   len =      370    nex =        1

     Sngl    29570    29939        +        0

>2618605        /39018

   len =     1334    nex =        0

>2618605        /12152

   len =     1346    nex =        0

>2618605        /11651

   len =     1166    nex =        1

     Sngl    47145    48310        +        0

>2618605        /8440

   len =     1831    nex =        7
```

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```
     Term   55825   55588          -        0
     Intr   56233   56081          -        0
     Intr   56458   56347          -        0
     Intr   56617   56546          -        0
     Intr   56796   56703          -        0
     Intr   57030   56879          -        0
     Init   57418   57264          -        0

>2618677        /14480

    len =    1296   nex =        3

     Term   12884   12633          -        0
     Intr   13526   13383          -        0
     Init   13928   13599          -        0

>2618677        /7573

    len =    1708   nex =        5

     Term   28265   27997          -        0
     Intr   28700   28345          -        0
     Intr   29322   28785          -        0
     Intr   29506   29408          -        0
     Init   29704   29600          -        0

>2618677        /111449

    len =     748   nex =        1

     Sngl   38479   37732          -        0

>2618677        /30006

    len =    2334   nex =       12

     Init   49398   49629          +        0
     Intr   49719   49823          +        0
     Intr   49924   49986          +        0
     Intr   50096   50129          +        0
     Intr   50217   50339          +        0
     Intr   50414   50476          +        0
     Intr   50565   50658          +        0
     Intr   50755   50800          +        0
     Intr   50894   50961          +        0
     Intr   51110   51158          +        0
     Intr   51254   51319          +        0
     Term   51417   51731          +        0

>2618677        /30716

    len =     804   nex =        1

     Sngl    5723    6526          +        0

>2618677        /29451

    len =     850   nex =        1

     Sngl   61457   62306          +        0
```

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```
>2618677       /141763

   len =     915   nex =       1

    Sngl    6460    5546         -        0

>2618677       /18592

   len =     342   nex =       1

    Sngl   76241   76582         +        0

>2618677       /452

   len =    1630   nex =       6

    Term   88176   87859         -        0
    Intr   88357   88285         -        0
    Intr   88547   88476         -        0
    Intr   88713   88642         -        0
    Intr   88988   88858         -        0
    Init   89480   89128         -        0

>2618677       /5532

   len =     915   nex =       1

    Sngl   89616   90530         +        0

>2618683       /37276

   len =    1974   nex =       7

    Term   24726   24402         -        0
    Intr   24881   24816         -        0
    Intr   25061   24963         -        0
    Intr   25435   25239         -        0
    Intr   25623   25576         -        0
    Intr   25828   25711         -        0
    Init   26375   26175         -        0

>2618683       /40508

   len =    2051   nex =       7

    Term   24726   24397         -        0
    Intr   24881   24816         -        0
    Intr   25061   24963         -        0
    Intr   25435   25239         -        0
    Intr   25623   25576         -        0
    Intr   25828   25711         -        0
    Init   26447   26175         -        0

>2618683       /13347

   len =    2662   nex =       6

    Init   27061   27168         +        0
    Intr   27764   28346         +        0
```

```
                                             1000
        Intr   28448    28669        +          0
        Intr   28748    28995        +          0
        Intr   29088    29227        +          0
        Term   29422    29722        +          0

    >2618683       /2657

       len =     2247    nex =       7

        Term   56579    56243        -          0
        Intr   56748    56672        -          0
        Intr   56919    56831        -          0
        Intr   57186    57056        -          0
        Intr   57409    57280        -          0
        Intr   57542    57518        -          0
        Init   58489    57939        -          0

    >2618683       /2085

       len =      561    nex =       2

        Init   64780    64842        +          0
        Term   64924    65340        +          0

    >2618683       /35456

       len =     1030    nex =       1

        Sngl   68721    69748        +          0

    >2623294       /6610

       len =     1969    nex =       2

        Init   13412    14121        +          0
        Term   14135    14416        +          0

    >2623294       /11739

       len =      521    nex =       2

        Init   27204    27453        +          0
        Term   27544    27724        +          0

    >2623294       /1297

       len =     1365    nex =       3

        Init   52988    53175        +          0
        Intr   53533    53759        +          0
        Term   53848    54135        +          0

    >2623294       /2337

       len =     4786    nex =       4

        Init   65365    65544        +          0
        Intr   65952    66570        +          0
        Intr   66666    66939        +          0
        Term   67114    67245        +          0
```

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```
>2623294        /30905

   len =     984   nex =       2

    Init   71769   71892         +        0
    Term   72257   72752         +        0

>2642152        /105724

   len =    1015   nex =       0

>2642152        /5605

   len =    1069   nex =       1

    Sngl   12014   12116         +        0

>2642152        /123288

   len =     600   nex =       2

    Term   13599   13304         -        0
    Init   13903   13724         -        0

>2642152        /18133

   len =     271   nex =       1

    Sngl   21652   21382         -        0

>2642152        /8256

   len =    3019   nex =       4

    Init   25623   26134         +        0
    Intr   26620   26968         +        0
    Intr   27055   27331         +        0
    Term   28305   28641         +        0

>2642152        /37775

   len =    2269   nex =       4

    Init   31242   31353         +        0
    Intr   32126   32188         +        0
    Intr   32277   32516         +        0
    Term   32603   33510         +        0

>2642152        /111626

   len =     250   nex =       1

    Sngl   33270   33510         +        0

>2642152        /27496

   len =    2548   nex =       9

    Init   33756   33970         +        0
```

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```
  Intr   34189   34217        +       0
  Intr   34335   34408        +       0
  Intr   34530   34631        +       0
  Intr   34670   34777        +       0
  Intr   35009   35100        +       0
  Intr   35365   35526        +       0
  Intr   35796   35880        +       0
  Term   35999   36303        +       0

>2642152       /39203

 len =     1168   nex =       3

  Init   39520   39810        +       0
  Intr   39958   40298        +       0
  Term   40402   40687        +       0

>2642152       /17221

 len =     2364   nex =      13

  Term   41130   41068        -       0
  Intr   41304   41234        -       0
  Intr   41463   41397        -       0
  Intr   41617   41549        -       0
  Intr   41866   41716        -       0
  Intr   42123   42048        -       0
  Intr   42323   42220        -       0
  Intr   42505   42409        -       0
  Intr   42680   42594        -       0
  Intr   42849   42763        -       0
  Intr   43018   42929        -       0
  Intr   43229   43152        -       0
  Init   43431   43342        -       0

>2642152       /158481

 len =     2590   nex =       6

  Term   52943   52448        -       0
  Intr   53435   53162        -       0
  Intr   53934   53766        -       0
  Intr   54202   54122        -       0
  Intr   54359   54302        -       0
  Init   55032   54833        -       0

>2642152       /114221

 len =      321   nex =       1

  Sngl   69754   70074        +       0

>2642427       /28853

 len =     1030   nex =       2

  Term  106334  106067        -       0
  Init  107087  106553        -       0

>2642427       /30636
```

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```
    len =     1167    nex =       3

     Term   25457   25117          -        0
     Intr   25685   25560          -        0
     Init   26283   25787          -        0

>2642427        /38686

    len =     1419    nex =       3

     Term   25457   25104          -        0
     Intr   25685   25560          -        0
     Init   26522   25787          -        0

>2642427        /25204

    len =     1692    nex =       3

     Term   32565   32226          -        0
     Intr   32850   32670          -        0
     Init   33917   33379          -        0

>2642427        /40307

    len =     1118    nex =       1

     Sngl   45533   46650          +        0

>2642427        /40943

    len =      631    nex =       1

     Sngl   48222   47592          -        0

>2642427        /22551

    len =      670    nex =       2

     Term    5656    5170          -        0
     Init    5836    5750          -        0

>2642427        /22936

    len =     3101    nex =       9

     Term    5656    5284          -        0
     Intr    5932    5750          -        0
     Intr    6183    6016          -        0
     Intr    6419    6285          -        0
     Intr    6590    6501          -        0
     Intr    6761    6684          -        0
     Intr    7515    6859          -        0
     Intr    7718    7600          -        0
     Init    8384    7931          -        0

>2645198        /28033

    len =      817    nex =       1
```

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```
    Sngl   15766   16582        +        0

>2645198       /144393

   len =      651   nex =       2

    Term   43394   43065        -        0
    Init   43715   43483        -        0

>2645198       /34660

   len =      676   nex =       2

    Term   51571   51243        -        0
    Init   51918   51697        -        0

>2645198       /21053

   len =      691   nex =       3

    Term   54371   54189        -        0
    Intr   54602   54463        -        0
    Init   54879   54688        -        0

>2645198       /36567

   len =     1873   nex =       2

    Term    6681    6341        -        0
    Init    8213    7815        -        0

>2651294       /6884

   len =     1979   nex =       8

    Init     119     467        +        0
    Intr     563     788        +        0
    Intr     984    1065        +        0
    Intr    1166    1223        +        0
    Intr    1300    1388        +        0
    Intr    1482    1577        +        0
    Intr    1671    1693        +        0
    Term    1814    2097        +        0

>2651294       /99409

   len =     1607   nex =       3

    Init   12703   12929        +        0
    Intr   13029   13341        +        0
    Term   13833   14309        +        0

>2651294       /19516

   len =     1380   nex =       4

    Init   17001   17093        +        0
    Intr   17192   17276        +        0
    Intr   17399   17532        +        0
    Term   17965   18220        +        0
```

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```
>2651294        /25574

   len =      610    nex =        1

     Sngl   29291   28700          -         0

>2651294        /15024

   len =      695    nex =        1

     Sngl   35758   35064          -         0

>2651294        /28121

   len =     1330    nex =        4

     Term   43723   43497          -         0
     Intr   44224   44091          -         0
     Intr   44474   44390          -         0
     Init   44667   44575          -         0

>2651294        /120147

   len =      792    nex =        1

     Sngl   48787   48117          -         0

>2651294        /12956

   len =     1078    nex =        1

     Sngl   55272   56349          +         0

>2651294        /97343

   len =     1233    nex =        3

     Init   59647   59727          +         0
     Intr   59886   60144          +         0
     Term   60429   60879          +         0

>2651294        /35274

   len =     3115    nex =       15

     Init   80840   81063          +         0
     Intr   81295   81464          +         0
     Intr   81548   81599          +         0
     Intr   81692   81717          +         0
     Intr   81829   81912          +         0
     Intr   82014   82124          +         0
     Intr   82222   82304          +         0
     Intr   82425   82515          +         0
     Intr   82617   82668          +         0
     Intr   82768   82858          +         0
     Intr   82979   83101          +         0
     Intr   83207   83329          +         0
     Intr   83465   83531          +         0
     Intr   83626   83705          +         0
     Term   83788   83954        +        0

>2656024       /2925

   len =     2110   nex =       6

     Init   21471   21726        +        0
     Intr   21825   21900        +        0
     Intr   21980   22075        +        0
     Intr   22220   22557        +        0
     Intr   23046   23175        +        0
     Term   23299   23578        +        0

>2656024       /10982

   len =     2112   nex =       8

     Term   36311   35994        -        0
     Intr   36483   36427        -        0
     Intr   36652   36568        -        0
     Intr   36876   36751        -        0
     Intr   37003   36960        -        0
     Intr   37209   37090        -        0
     Intr   37445   37291        -        0
     Init   38105   38035        -        0

>2656024       /115913

   len =      454   nex =       1

     Sngl   69260   69713        +        0

>2656025       /42551

   len =     1597   nex =       3

     Init   21954   22111        +        0
     Intr   22318   22630        +        0
     Term   22710   23356        +        0

>2656025       /17690

   len =     2548   nex =       6

     Init   26822   27289        +        0
     Intr   27502   27719        +        0
     Intr   27833   27945        +        0
     Intr   28683   28746        +        0
     Intr   28894   28947        +        0
     Term   29171   29369        +        0

>2656025       /31580

   len =     2657   nex =       3

     Term   34185   33154        -        0
     Intr   34744   34283        -        0
     Init   35810   35427        -        0

>2656025       /21044
```

```
    len =     4643    nex =       16

     Term    36770    36474          -        0
     Intr    36904    36853          -        0
     Intr    37063    37002          -        0
     Intr    37298    37211          -        0
     Intr    37535    37467          -        0
     Intr    37706    37649          -        0
     Intr    37887    37786          -        0
     Intr    38077    37976          -        0
     Intr    38499    38169          -        0
     Intr    38817    38701          -        0
     Intr    38980    38912          -        0
     Intr    39610    39366          -        0
     Intr    40211    39972          -        0
     Intr    40458    40363          -        0
     Intr    40839    40723          -        0
     Init    41116    40940          -        0

>2656026        /27668

    len =      641    nex =        1

     Sngl    21786    22426          +        0

>2656026        /32253

    len =     1708    nex =        2

     Init    22982    24225          +        0
     Term    24310    24689          +        0

>2656026        /39882

    len =     1499    nex =        2

     Init    22997    24225          +        0
     Term    24310    24471          +        0

>2656026        /205741

    len =     1213    nex =        4

     Init    33785    34004          +        0
     Intr    34198    34304          +        0
     Intr    34400    34430          +        0
     Term    34537    34990          +        0

>2656026        /27452

    len =     1064    nex =        1

     Sngl    39160    40223          +        0

>2656026        /34082

    len =      773    nex =        1

     Sngl    47232    46460          -        0
```

```
>2656026        /42826

   len =    1690   nex =       3

     Init   47575   48443        +        0
     Intr   48536   48674        +        0
     Term   48854   49260        +        0

>2656026        /9664

   len =     810   nex =       1

     Sngl   50539   49730        -        0

>2656026        /16760

   len =    3571   nex =       0

>2656027        /37474

   len =    5143   nex =       4

     Init   22714   22946        +        0
     Intr   23554   23642        +        0
     Intr   23726   23824        +        0
     Term   23923   24012        +        0

>2656027        /117516

   len =    1426   nex =       4

     Init   22758   22946        +        0
     Intr   23554   23642        +        0
     Intr   23726   23824        +        0
     Term   23923   24183        +        0

>2656027        /96557

   len =    1396   nex =       4

     Init   22776   22946        +        0
     Intr   23554   23642        +        0
     Intr   23726   23824        +        0
     Term   23923   24171        +        0

>2656027        /34532

   len =    2155   nex =       4

     Init   24706   25078        +        0
     Intr   25689   26087        +        0
     Intr   26183   26517        +        0
     Term   26605   26860        +        0

>2656028        /39718

   len =     910   nex =       2

     Init   21537   21782        +        0
```

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```
    Term   22349   22446        +       0

>2656028       /11128

   len =    1591   nex =       5

    Init   21555   21782        +       0
    Intr   22349   22446        +       0
    Intr   22555   22722        +       0
    Intr   22802   22844        +       0
    Term   22931   23145        +       0

>2656028       /38185

   len =    2448   nex =       8

    Term   23351   23070        -       0
    Intr   23563   23476        -       0
    Intr   23838   23740        -       0
    Intr   24026   23922        -       0
    Intr   24195   24126        -       0
    Intr   24373   24277        -       0
    Intr   24640   24499        -       0
    Init   25228   25069        -       0

>2656028       /27013

   len =    2579   nex =       9

    Term   23351   23054        -       0
    Intr   23563   23476        -       0
    Intr   23838   23740        -       0
    Intr   24026   23922        -       0
    Intr   24195   24126        -       0
    Intr   24373   24277        -       0
    Intr   24640   24499        -       0
    Intr   25228   25069        -       0
    Init   25632   25564        -       0

>2656028       /28596

   len =    2454   nex =      10

    Term   39978   39624        -       0
    Intr   40161   40113        -       0
    Intr   40298   40244        -       0
    Intr   40511   40391        -       0
    Intr   40705   40604        -       0
    Intr   40916   40809        -       0
    Intr   41213   40996        -       0
    Intr   41361   41289        -       0
    Intr   41523   41447        -       0
    Init   42077   41767        -       0
>2656028       /103642

   len =     690   nex =       1

    Sngl   55512   55324        -       0

>2656028       /36094
```

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```
        len =     2124    nex =        3

         Init   56165    56791          +         0
         Intr   56822    56893          +         0
         Term   57886    58288          +         0

  >2656028         /3303

        len =     1667    nex =        4

         Term   58518    58274          -         0
         Intr   58878    58601          -         0
         Intr   59124    58950          -         0
         Init   59940    59506          -         0

  >2656028         /18435

        len =     2060    nex =        8

         Init   60472    60847          +         0
         Intr   60939    61071          +         0
         Intr   61351    61440          +         0
         Intr   61533    61657          +         0
         Intr   61748    61790          +         0
         Intr   61872    61989          +         0
         Intr   62089    62138          +         0
         Term   62218    62531          +         0

  >2656028         /11539

        len =      864    nex =        1

         Sngl   65472    66335          +         0

  >2656028         /34900

        len =      430    nex =        1

         Sngl   75838    76262          +         0

  >2656028         /16977

        len =      430    nex =        1

         Sngl   75840    76262          +         0

  >2656028         /155377

        len =      850    nex =        5

         Init     9258     9512         +         0
         Intr     9598     9659         +         0
         Intr     9739     9790         +         0
         Intr     9876     9990         +         0
         Term    10076    10107         +         0

  >2656029         /2641

        len =     1789    nex =        5
```

```
     Init    15938    16144          +        0
     Intr    16242    16362          +        0
     Intr    16458    16549          +        0
     Intr    16632    16728          +        0
     Term    16810    17371          +        0

>2656029        /1843

   len =       670    nex =        1

     Sngl    18752    18085          -        0

>2656029        /578

   len =      1630    nex =        7

     Init    36717    36759          +        0
     Intr    36898    36971          +        0
     Intr    37133    37178          +        0
     Intr    37268    37338          +        0
     Intr    37454    37498          +        0
     Intr    37595    37663          +        0
     Term    37791    38345          +        0

>2656029        /22827

   len =       884    nex =        1

     Sngl      374     1257          +        0

>2656029        /5589

   len =      1459    nex =        3

     Term    43267    42861          -        0
     Intr    43429    43348          -        0
     Init    43780    43535          -        0

>2656029        /30314

   len =       915    nex =        1

     Sngl    48635    49549          +        0

>2656029        /38273

   len =      3043    nex =        4

     Init     6585     6905          +        0
     Intr     7927     8060          +        0
     Intr     8561     8708          +        0
     Term     9213     9319          +        0

>2656029        /10991

   len =       550    nex =        1

     Sngl    66286    66832          +        0
```

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```
>2656030       /42911

   len =     933   nex =        3

     Init   78500   78616         +        0
     Intr   78721   78891         +        0
     Term   79064   79432         +        0

>2656030       /5331

   len =    1630   nex =        5

     Term   79640   79440         -        0
     Intr   79934   79737         -        0
     Intr   80170   80021         -        0
     Intr   80795   80709         -        0
     Init   81069   80880         -        0

>2656031       /6333

   len =    1318   nex =        2

     Init   18702   19086         +        0
     Term   19654   20019         +        0

>2656032       /31040

   len =    3269   nex =       10

     Init   17542   17641         +        0
     Intr   17886   18084         +        0
     Intr   18311   18413         +        0
     Intr   18505   18575         +        0
     Intr   18958   19119         +        0
     Intr   19218   19355         +        0
     Intr   19444   19737         +        0
     Intr   19841   19993         +        0
     Intr   20110   20205         +        0
     Term   20311   20555         +        0

>2656032       /1048

   len =    1073   nex =        1

     Sngl   34012   32940         -        0

>2660661       /31042

   len =    1330   nex =        1

     Sngl   12325   13650         +        0

>2660661       /1383

   len =    1030   nex =        2

     Term   64341   63993         -        0
     Init   65022   64911         -        0

>2660661       /39167
```

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```
       len =     1410   nex =       3

        Init   77572   77856         +        0
        Intr   77938   78144         +        0
        Term   78228   78610         +        0

  >2660661         /4029

       len =     2474   nex =       8

        Term   78971   78641         -        0
        Intr   79182   79069         -        0
        Intr   79367   79265         -        0
        Intr   79521   79457         -        0
        Intr   79694   79606         -        0
        Intr   79951   79775         -        0
        Intr   80178   80029         -        0
        Init   81114   80933         -        0

  >2673901         /12267

       len =     1283   nex =       1

        Sngl   21553   21782         +        0

  >2673901         /33002

       len =     2397   nex =       3

        Init   25887   26600         +        0
        Intr   27248   27393         +        0
        Term   27470   28283         +        0

  >2673901         /28982

       len =     1630   nex =       2

        Init   29263   29613         +        0
        Term   30084   30468         +        0

  >2673901         /32748

       len =     3159   nex =       9

        Term   32389   32054         -        0
        Intr   32585   32474         -        0
        Intr   32774   32682         -        0
        Intr   33085   32882         -        0
        Intr   33504   33364         -        0
        Intr   33696   33592         -        0
        Intr   34178   33798         -        0
        Intr   34443   34303         -        0
        Init   35212   34776         -        0

  >2673901         /38748

       len =      235   nex =       1

        Sngl   35473   35707         +        0
```

```
>2689438        /151406

   len =      472    nex =        1

    Sngl   16845   17316          +         0

>2689438        /3000

   len =     2973    nex =        4

    Term   25402   24983          -         0
    Intr   26284   26031          -         0
    Intr   27230   26975          -         0
    Init   27948   27691          -         0

>2689438        /31290

   len =     1945    nex =        4

    Init   48660   48849          +         0
    Intr   48947   49173          +         0
    Intr   49650   49983          +         0
    Term   50177   50604          +         0

>2689438        /25158

   len =      448    nex =        1

    Sngl   50196   50643          +         0

>2689438        /35916

   len =     2140    nex =        8

    Term   72752   72356          -         0
    Intr   72927   72844          -         0
    Intr   73134   73078          -         0
    Intr   73359   73231          -         0
    Intr   73775   73689          -         0
    Intr   73908   73861          -         0
    Intr   74175   74019          -         0
    Init   74495   74380          -         0

>2696018        /143114

   len =      250    nex =        1

    Sngl   24191   24437          +         0

>2696018        /5698

   len =     1017    nex =        1

    Sngl   32527   32321          -         0

>2696018        /92795

   len =      654    nex =        1
```

```
    Sngl   49709   49056        -       0

>2696018       /6629

   len =    2745   nex =       8

    Init   56009   56062        +       0
    Intr   56175   56325        +       0
    Intr   56866   56932        +       0
    Intr   57026   57174        +       0
    Intr   57438   57571        +       0
    Intr   57657   57755        +       0
    Intr   57849   57924        +       0
    Term   58005   58355        +       0

>2696018       /11275

   len =    2084   nex =       9

    Init   56016   56062        +       0
    Intr   56175   56325        +       0
    Intr   56866   56932        +       0
    Intr   57026   57174        +       0
    Intr   57370   57405        +       0
    Intr   57438   57571        +       0
    Intr   57657   57755        +       0
    Intr   57849   57924        +       0
    Term   58005   58099        +       0

>2696018       /4330

   len =    1842   nex =       6

    Term   67677   67445        -       0
    Intr   67827   67768        -       0
    Intr   68176   68136        -       0
    Intr   68784   68715        -       0
    Intr   68912   68859        -       0
    Init   69286   69237        -       0

>2696018       /37111

   len =     992   nex =       2

    Term   79220   78599        -       0
    Init   79576   79309        -       0

>2702261       /26812

   len =    2400   nex =       7

    Init    2306    2536        +       0
    Intr    2938    2981        +       0
    Intr    3093    3250        +       0
    Intr    3640    3767        +       0
    Intr    3879    4001        +       0
    Intr    4101    4220        +       0
    Term    4310    4705        +       0

>2702261       /3972
```

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```
  len =     2490    nex =       8

   Init     2306     2536          +          0
   Intr     2938     2981          +          0
   Intr     3093     3250          +          0
   Intr     3422     3557          +          0
   Intr     3640     3767          +          0
   Intr     3879     4001          +          0
   Intr     4101     4220          +          0
   Term     4310     4795          +          0

>2702261        /38147

  len =     2387    nex =       6

   Init    23695    23892          +          0
   Intr    24225    24350          +          0
   Intr    24510    24614          +          0
   Intr    24888    24956          +          0
   Intr    25075    25188          +          0
   Term    25563    26081          +          0

>2702261        /18783

  len =     3623    nex =       7

   Init    85480    85974          +          0
   Intr    86065    86230          +          0
   Intr    86316    86366          +          0
   Intr    86456    86638          +          0
   Intr    86718    86811          +          0
   Intr    86890    87554          +          0
   Term    87651    87783          +          0

>2708736        /18625

  len =     1123    nex =       2

   Term    21014    20394          -          0
   Init    21516    21326          -          0

>2708736        /18284

  len =      733    nex =       1

   Sngl     2752     2020          -          0

>2708736        /33031

  len =      509    nex =       2

   Init    39554    39695          +          0
   Term    39767    40041          +          0

>2708736        /22711

  len =      766    nex =       1

   Sngl    56611    57376          +          0
```

```
>2708736        /12534

   len =      808    nex =       1

     Sngl   57189   56382         -        0

>2708736        /24747

   len =     1231    nex =       1

     Sngl   59076   60306         +        0

>2708736        /9676

   len =      895    nex =       4

     Init   63519   63714         +        0
     Intr   63796   63961         +        0
     Intr   64080   64133         +        0
     Term   64232   64413         +        0

>2708736        /40806

   len =     2170    nex =       4

     Init   66493   67471         +        0
     Intr   67805   67971         +        0
     Intr   68059   68143         +        0
     Term   68241   68655         +        0

>2739359        /22595

   len =     2809    nex =       5

     Term   29529   28886         -        0
     Intr   30066   29793         -        0
     Intr   30458   30289         -        0
     Intr   30730   30590         -        0
     Init   30933   30852         -        0

>2739359        /9368

   len =      715    nex =       2

     Init   55832   55932         +        0
     Term   56029   56546         +        0

>2739359        /30772

   len =      673    nex =       1

     Sngl   55861   55932         +        0

>2739359        /14528

   len =      644    nex =       1

     Sngl   56029   56527         +        0
```

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```
>2739359        /31774

   len =     2950    nex =       12

    Term     3431     3158          -         0
    Intr     3534     3459          -         0
    Intr     3857     3781          -         0
    Intr     4371     4277          -         0
    Intr     4756     4623          -         0
    Intr     4924     4846          -         0
    Intr     5064     4995          -         0
    Intr     5348     5159          -         0
    Intr     5522     5428          -         0
    Intr     5694     5605          -         0
    Intr     5801     5770          -         0
    Init     6098     5900          -         0

>2739359        /628

   len =      827    nex =        1

    Sngl    65777    66603          +         0

>2739359        /33680

   len =      634    nex =        1

    Sngl    69683    69050          -         0

>2739359        /1999

   len =     1979    nex =        6

    Term    79655    79490          -         0
    Intr    79924    79752          -         0
    Intr    80307    80005          -         0
    Intr    80697    80536          -         0
    Intr    81152    80784          -         0
    Init    81468    81236          -         0

>2749918        /27548

   len =     1572    nex =        4

    Term   118910   118818          -         0
    Intr   119099   119049          -         0
    Intr   119342   119186          -         0
    Init   119799   119495          -         0

>2749918        /2157

   len =     1164    nex =        5

    Term    33097    32732          -         0
    Intr    33226    33170          -         0
    Intr    33418    33313          -         0
    Intr    33578    33520          -         0
    Init    33895    33656          -         0

>2749918        /41337
```

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```
      len =     2907   nex =        8

       Term   40133   39852          -         0
       Intr   40316   40251          -         0
       Intr   40452   40399          -         0
       Intr   40631   40560          -         0
       Intr   40820   40725          -         0
       Intr   41443   41366          -         0
       Intr   41920   41841          -         0
       Init   42758   42004          -         0

 >2749918        /5055

      len =     2249   nex =        8

       Term   73192   72956          -         0
       Intr   73427   73285          -         0
       Intr   73554   73513          -         0
       Intr   73679   73638          -         0
       Intr   73857   73758          -         0
       Intr   74009   73942          -         0
       Intr   74203   74119          -         0
       Init   75204   74990          -         0

 >2760164        /122489

      len =      748   nex =        3

       Term    1929    1667          -         0
       Intr    2065    2000          -         0
       Init    2414    2235          -         0

 >2760164        /141953

      len =      404   nex =        1

       Sngl   40494   40091          -         0

 >2760164        /6393

      len =     1887   nex =        4

       Term   45207   44626          -         0
       Intr   45909   45643          -         0
       Intr   46332   46110          -         0
       Init   46512   46432          -         0

 >2760164        /12030

      len =     1175   nex =        1

       Sngl   62893   63617          +         0

 >2760164        /5684

      len =     1901   nex =        6

       Init   71453   71775          +         0
       Intr   71875   71990          +         0
```

```
    Intr   72555   72678        +        0
    Intr   72782   72930        +        0
    Intr   73031   73093        +        0
    Term   73185   73353        +        0

>2760164        /36046

    len =      910   nex =        3

    Init     871    1095        +        0
    Intr    1189    1297        +        0
    Term    1413    1767        +        0

>2760164        /6826

    len =      758   nex =        3

    Init     991    1095        +        0
    Intr    1189    1297        +        0
    Term    1413    1748        +        0

>2760165        /19114

    len =     1646   nex =        1

    Sngl   20275   19212        -        0

>2760165        /4193

    len =     1620   nex =        5

    Init   30702   30854        +        0
    Intr   30939   31019        +        0
    Intr   31132   31326        +        0
    Intr   31420   31797        +        0
    Term   31892   32219        +        0

>2760165        /17486

    len =      386   nex =        1

    Sngl   32921   32536        -        0

>2760165        /17455

    len =     1330   nex =        4

    Term   32949   32576        -        0
    Intr   33144   33034        -        0
    Intr   33293   33222        -        0
    Init   33897   33392        -        0

>2760165        /2869

    len =     2553   nex =        6

    Term   32949   32636        -        0
    Intr   33144   33034        -        0
    Intr   33293   33222        -        0
    Intr   34521   33392        -        0
```

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```
     Intr   34888   34819         -         0
     Init   35188   34989         -         0

>2760165        /1022

    len =    1103   nex =        3

     Term   37453   37053         -         0
     Intr   37627   37537         -         0
     Init   38155   37697         -         0

>2760165        /35974

    len =    1669   nex =        4

     Init   42735   42859         +         0
     Intr   43198   43462         +         0
     Intr   43554   43748         +         0
     Term   43823   44403         +         0

>2760165        /34861

    len =    2759   nex =        4

     Term   45311   44615         -         0
     Intr   45749   45508         -         0
     Intr   46640   46252         -         0
     Init   47373   47034         -         0

>2760165        /146274

    len =    1378   nex =        3

     Init   56343   56430         +         0
     Intr   56568   56695         +         0
     Term   56823   57131         +         0

>2760165        /2996

    len =    1590   nex =        3

     Init    6987    7404         +         0
     Intr    7510    8043         +         0
     Term    8152    8576         +         0

>2760166        /37308

    len =    1640   nex =        3

     Init   36323   36769         +         0
     Intr   36888   37165         +         0
     Term   37289   37962         +         0

>2760166        /37792

    len =    1517   nex =        2

     Init   36888   37165         +         0
     Term   37289   37987         +         0
```

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```
>2760166        /15419

   len =     762   nex =       2

    Term    5587    5255         -        0
    Init    6016    5725         -        0

>2760167        /6274

   len =    2810   nex =      11

    Term   32480   31948         -        0
    Intr   32689   32573         -        0
    Intr   32895   32777         -        0
    Intr   33101   32993         -        0
    Intr   33238   33200         -        0
    Intr   33451   33373         -        0
    Intr   33637   33563         -        0
    Intr   33811   33747         -        0
    Intr   33992   33937         -        0
    Intr   34225   34133         -        0
    Init   34757   34493         -        0

>2760167        /101988

   len =    1033   nex =       1

    Sngl   40367   40745         +        0

>2760167        /92179

   len =     993   nex =       1

    Sngl   40367   40745         +        0

>2760167        /13697

   len =    1356   nex =       3

    Term   42553   42287         -        0
    Intr   42795   42646         -        0
    Init   43642   43418         -        0

>2760167        /30674

   len =     804   nex =       3

    Term   44295   44028         -        0
    Intr   44536   44458         -        0
    Init   44831   44714         -        0

>2760167        /7119

   len =    1930   nex =       3

    Init   47503   47617         +        0
    Intr   47702   48982         +        0
    Term   49233   49428         +        0

>2760167        /9480
```

```
     len =     1375    nex =       6

      Init   51156   51210          +         0
      Intr   51501   51557          +         0
      Intr   51659   51776          +         0
      Intr   51994   52083          +         0
      Intr   52170   52349          +         0
      Term   52439   52514          +         0

>2760167        /35248

     len =      116    nex =       1

      Sngl   52672   52787          +         0

>2760167        /20790

     len =     1918    nex =       6

      Init   53351   53565          +         0
      Intr   53877   54048          +         0
      Intr   54137   54333          +         0
      Intr   54434   54601          +         0
      Intr   54690   54818          +         0
      Term   54954   55268          +         0

>2760167        /8984

     len =     3272    nex =      14

      Init    6342    6410          +         0
      Intr    6499    6570          +         0
      Intr    6719    6773          +         0
      Intr    6858    6876          +         0
      Intr    6960    7027          +         0
      Intr    7214    7288          +         0
      Intr    7382    7437          +         0
      Intr    7542    7586          +         0
      Intr    7667    7930          +         0
      Intr    8046    8140          +         0
      Intr    8260    8350          +         0
      Intr    8439    8549          +         0
      Intr    8647    8841          +         0
      Term    8935    9304          +         0

>2760167        /37493

     len =     3313    nex =       4

      Term   66659   66266          -         0
      Intr   68318   68043          -         0
      Intr   69011   68592          -         0
      Init   69578   69461          -         0

>2760167        /7548

     len =     1930    nex =       6

      Term   79732   79374          -         0
```

```
        Intr   80062   79829        -        0
        Intr   80336   80169        -        0
        Intr   80655   80426        -        0
        Intr   80848   80749        -        0
        Init   81298   80951        -        0

    >2760167        /10973

       len =     1259   nex =        1

        Sngl   82221   82174        -        0

    >2760167        /10749

       len =     1330   nex =        3

        Term   81820   81596        -        0
        Intr   82221   82174        -        0
        Init   82918   82706        -        0

    >2760168        /1165

       len =     1197   nex =        3

        Init   13720   13876        +        0
        Intr   14260   14352        +        0
        Term   14686   14916        +        0

    >2760168        /42577

       len =      796   nex =        2

        Term   23611   23112        -        0
        Init   23907   23692        -        0

    >2760168        /12729

       len =      216   nex =        1

        Sngl    2967    2752        -        0

    >2760168        /19343

       len =      612   nex =        1

        Sngl    2989    2378        -        0

    >2760168        /17242

       len =     2173   nex =        7

        Init   31582   31714        +        0
        Intr   31798   31982        +        0
        Intr   32205   32264        +        0
        Intr   32354   32635        +        0
        Intr   32868   32963        +        0
        Intr   33072   33268        +        0
        Term   33388   33754        +        0

    >2760168        /94968
    len =     467    nex =        1

     Sngl    3825    4291          +         0

>2760168        /152076

    len =    1210    nex =        3

     Init     598     955          +         0
     Intr    1255    1356          +         0
     Term    1441    1806          +         0

>2760169        /8993

    len =    2713    nex =       11

     Init       1     127          +         0
     Intr     474     621          +         0
     Intr     698     769          +         0
     Intr     943    1014          +         0
     Intr    1227    1298          +         0
     Intr    1396    1539          +         0
     Intr    1636    1716          +         0
     Intr    1844    1886          +         0
     Intr    1966    2037          +         0
     Intr    2143    2214          +         0
     Term    2426    2713          +         0

>2760169        /14492

    len =     651    nex =        2

     Init   20745   20918          +         0
     Term   21014   21373          +         0

>2760169        /5810

    len =    1439    nex =        1

     Sngl   24319   23980          -         0

>2760169        /38421

    len =    1851    nex =        1

     Sngl   44204   42354          -         0

>2760169        /16827

    len =     564    nex =        1

     Sngl   48497   49060          +         0

>2760169        /12970

    len =    1536    nex =        3

     Term   72431   71753          -         0
     Intr   72794   72517          -         0
```

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```
     Init    73288    72925         -        0

>2760170        /36309

   len =     2470    nex =        7

     Term     8439     7872         -        0
     Intr     8753     8655         -        0
     Intr     8923     8846         -        0
     Intr     9409     9327         -        0
     Intr     9622     9525         -        0
     Intr     9990     9959         -        0
     Init    10341    10081         -        0

>2760170        /100904

   len =      616    nex =        2

     Term    72185    72007         -        0
     Init    72622    72442         -        0

>2760170        /4536

   len =      706    nex =        2

     Term    72185    71972         -        0
     Init    72677    72442         -        0

>2760170        /292

   len =      684    nex =        2

     Term    72185    72004         -        0
     Init    72687    72442         -        0

>2760170        /99615

   len =     1994    nex =        5

     Init    79105    79274         +        0
     Intr    79891    79988         +        0
     Intr    80281    80424         +        0
     Intr    80520    80749         +        0
     Term    80836    81098         +        0

>2760171        /4585

   len =     2746    nex =       12

     Term    25736    25556         -        0
     Intr    25904    25816         -        0
     Intr    26088    25978         -        0
     Intr    26440    26311         -        0
     Intr    26630    26532         -        0
     Intr    26871    26767         -        0
     Intr    27087    26953         -        0
     Intr    27378    27174         -        0
     Intr    27618    27490         -        0
     Intr    27801    27711         -        0
     Intr    27896    27863         -        0
```

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```
   Init   28301   28131        -       0

>2760171      /12301

  len =    1349   nex =       4

   Init   51419   51657        +       0
   Intr   51763   51863        +       0
   Intr   51971   52167        +       0
   Term   52288   52767        +       0

>2760171      /31563

  len =    2637   nex =       4

   Init    7482    7708        +       0
   Intr    7821    7962        +       0
   Intr    8116    8177        +       0
   Term    8473    8676        +       0

>2760171      /37100

  len =    1735   nex =       8

   Init   69229   69386        +       0
   Intr   69531   69707        +       0
   Intr   69794   69925        +       0
   Intr   70009   70107        +       0
   Intr   70188   70304        +       0
   Intr   70399   70494        +       0
   Intr   70648   70760        +       0
   Term   70850   70954        +       0

>2760172      /24599

  len =     730   nex =       4

   Term   26279   26046        -       0
   Intr   26489   26370        -       0
   Intr   26593   26564        -       0
   Init   26767   26693        -       0

>2760172      /25884

  len =     670   nex =       1

   Sngl   51002   51664        +       0

>2760172      /8981

  len =     357   nex =       1

   Sngl   51004   51353        +       0

>2760172      /21903

  len =     940   nex =       1

   Sngl   57422   58361        +       0
```

```
>2760172        /14033

   len =     1585    nex =        4

    Term    71969    71571          -         0
    Intr    72172    72083          -         0
    Intr    72354    72271          -         0
    Init    73155    72644          -         0

>2760172        /205721

   len =      693    nex =        1

    Sngl    78960    78268          -         0

>2760173        /41408

   len =     1404    nex =        3

    Init     2606     2664          +         0
    Intr     2991     3372          +         0
    Term     3572     4009          +         0

>2760173        /100590

   len =     1692    nex =        5

    Init    27003    27364          +         0
    Intr    27452    27570          +         0
    Intr    27661    27790          +         0
    Intr    27912    28130          +         0
    Term    28473    28694          +         0

>2760173        /14898

   len =     1241    nex =        3

    Term    43600    43275          -         0
    Intr    44232    44110          -         0
    Init    44515    44319          -         0

>2760173        /7791

   len =     1186    nex =        3

    Term    43600    43330          -         0
    Intr    44232    44110          -         0
    Init    44515    44319          -         0

>2760173        /15331

   len =     1000    nex =        3

    Term    43600    43516          -         0
    Intr    44232    44110          -         0
    Init    44515    44319          -         0

>2760173        /4734

   len =     1047    nex =        2
```

```
     Init     9992    10398          +         0
     Term    10735    11038          +         0

>2760316        /42320

   len =     1227    nex =        2

     Init    13777    14194          +         0
     Term    14314    15003          +         0

>2760316        /14043

   len =     1172    nex =        2

     Init    13832    14194          +         0
     Term    14314    15003          +         0

>2760316        /28426

   len =     1303    nex =        2

     Term    15264    15087          -         0
     Init    15991    15736          -         0

>2760316        /1542

   len =     1358    nex =        2

     Term    15264    15084          -         0
     Init    15991    15736          -         0

>2760316        /7553

   len =     1150    nex =        1

     Sngl    36870    38015          +         0

>2760316        /13346

   len =     2352    nex =        9

     Init    61238    61435          +         0
     Intr    61585    61780          +         0
     Intr    61871    61995          +         0
     Intr    62136    62200          +         0
     Intr    62323    62535          +         0
     Intr    62670    62717          +         0
     Intr    62833    62940          +         0
     Intr    63036    63176          +         0
     Term    63264    63589          +         0

>2760316        /27632

   len =     1338    nex =        4

     Init    86833    87026          +         0
     Intr    87327    87427          +         0
     Intr    87723    87820          +         0
     Term    87960    88170          +         0
```

```
>2760829        /33692

   len =     1822    nex =        3

     Init    11711    12231          +        0
     Intr    12887    13110          +        0
     Term    13186    13532          +        0

>2760829        /41755

   len =     1546    nex =        2

     Term    14354    13832          -        0
     Init    15377    14681          -        0

>2760829        /28786

   len =     1722    nex =        1

     Sngl       70     1791          +        0

>2760829        /4754

   len =      970    nex =        2

     Term    77740    77286          -        0
     Init    77876    77815          -        0

>2795802        /17353

   len =     1734    nex =        3

     Term    20024    19317          -        0
     Intr    20663    20345          -        0
     Init    21050    20759          -        0

>2795802        /27620

   len =      670    nex =        1

     Sngl    32381    33046          +        0

>2815404        /42968

   len =      610    nex =        1

     Sngl    14332    14937          +        0

>2815404        /22971

   len =      620    nex =        1

     Sngl    14341    14960          +        0

>2815404        /9731

   len =      675    nex =        1

     Sngl    35713    35039          -        0
```

```
>2815404       /18451

   len =    2372   nex =       8

     Init   45075   45423         +        0
     Intr   45497   45617         +        0
     Intr   45704   46131         +        0
     Intr   46227   46383         +        0
     Intr   46695   46777         +        0
     Intr   46860   46956         +        0
     Intr   47066   47135         +        0
     Term   47312   47446         +        0

>2815404       /15499

   len =    1870   nex =       4

     Init   67484   67838         +        0
     Intr   68518   68746         +        0
     Intr   68868   68992         +        0
     Term   69083   69344         +        0

>2815404       /18800

   len =     910   nex =       2

     Init   70956   71114         +        0
     Term   71453   71857         +        0

>2815404       /9878

   len =     687   nex =       1

     Sngl   77572   76886         -        0

>2815519       /96891

   len =    1073   nex =       6

     Term   22493   22461         -        0
     Intr   22634   22576         -        0
     Intr   22953   22881         -        0
     Intr   23103   23039         -        0
     Intr   23313   23201         -        0
     Init   23533   23402         -        0

>2815519       /25380

   len =     750   nex =       1

     Sngl   26997   26248         -        0

>2815519       /8432

   len =     310   nex =       1

     Sngl   27005   26703         -        0

>2815519       /36424
```

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```
    len =     412    nex =        1

     Sngl   27008   26597          -         0

>2815519        /2327

    len =     394    nex =        1

     Sngl   27008   26615          -         0

>2815519        /113981

    len =     819    nex =        1

     Sngl   27010   26192          -         0

>2815519        /7630

    len =     763    nex =        1

     Sngl   27010   26248          -         0

>2815519        /31731

    len =     762    nex =        1

     Sngl   27010   26249          -         0

>2815519        /35047

    len =     396    nex =        1

     Sngl   27010   26615          -         0

>2815519        /16438

    len =     400    nex =        1

     Sngl   27014   26615          -         0

>2815519        /36235

    len =     773    nex =        1

     Sngl   27020   26248          -         0

>2815519        /20963

    len =    2032    nex =        9

     Init   44481   44658          +         0
     Intr   44737   44883          +         0
     Intr   44989   45105          +         0
     Intr   45281   45424          +         0
     Intr   45505   45591          +         0
     Intr   45673   45788          +         0
     Intr   45891   46072          +         0
     Intr   46172   46229          +         0
     Term   46318   46512          +         0
```

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```
>2815519        /4249

   len =    1602   nex =        5

     Init   47167   47260         +        0
     Intr   47353   47634         +        0
     Intr   47724   47945         +        0
     Intr   48032   48196         +        0
     Term   48281   48768         +        0

>2815519        /11560

   len =    1777   nex =        4

     Init   61717   61829         +        0
     Intr   61954   62030         +        0
     Intr   62992   63183         +        0
     Term   63276   63493         +        0

>2815519        /113946

   len =     504   nex =        2

     Init   62991   63183         +        0
     Term   63276   63494         +        0

>2815519        /36710

   len =     414   nex =        1

     Sngl   76124   75711         -        0

>2815519        /14108

   len =    1046   nex =        1

     Sngl   76756   75711         -        0

>2815519        /38663

   len =    1041   nex =        1

     Sngl   76756   75716         -        0

>2815519        /11318

   len =    1438   nex =        2

     Term   76754   75679         -        0
     Init   77108   77052         -        0

>2815519        /38261

   len =    1484   nex =        2

     Term   76754   75635         -        0
     Init   77108   77052         -        0

>2815519        /25772
```

```
      len =     1427    nex =        1

       Sngl   77108    77052          -         0

>2815519        /6957

      len =     2110    nex =        5

       Init   89813    89995          +         0
       Intr   90317    90488          +         0
       Intr   90587    91007          +         0
       Intr   91091    91156          +         0
       Term   91508    91915          +         0

>2815519        /32361

      len =     2950    nex =       12

       Term   92911    92505          -         0
       Intr   93238    92998          -         0
       Intr   93386    93324          -         0
       Intr   93536    93468          -         0
       Intr   93725    93642          -         0
       Intr   94016    93811          -         0
       Intr   94196    94106          -         0
       Intr   94389    94285          -         0
       Intr   94796    94722          -         0
       Intr   94970    94878          -         0
       Intr   95139    95064          -         0
       Init   95447    95254          -         0

>2827513        /34120

      len =     2154    nex =        3

       Term   16992    16242          -         0
       Intr   17554    17075          -         0
       Init   18395    18232          -         0

>2827513        /24334

      len =     1775    nex =        3

       Term   23483    22952          -         0
       Intr   24280    23812          -         0
       Init   24726    24647          -         0

>2827513        /39740

      len =     2470    nex =        7

       Init   26839    26915          +         0
       Intr   27027    27083          +         0
       Intr   27164    27220          +         0
       Intr   27310    27350          +         0
       Intr   27467    27538          +         0
       Intr   27635    27714          +         0
       Term   27850    28215          +         0
```

```
>2827513        /24184

   len =     190   nex =       1

     Sngl   28607   28793         +        0

>2827513        /229

   len =    2269   nex =       8

     Init   28607   28791         +        0
     Intr   28967   29043         +        0
     Intr   29236   29291         +        0
     Intr   29386   29458         +        0
     Intr   29960   30071         +        0
     Intr   30213   30286         +        0
     Intr   30393   30467         +        0
     Term   30566   30875         +        0

>2827513        /39364

   len =     402   nex =       1

     Sngl   42615   43016         +        0

>2827513        /27911

   len =    4030   nex =      12

     Init   45284   45583         +        0
     Intr   45967   46094         +        0
     Intr   46215   46286         +        0
     Intr   46380   46493         +        0
     Intr   46958   47026         +        0
     Intr   47094   47150         +        0
     Intr   47288   47341         +        0
     Intr   47427   47489         +        0
     Intr   47586   47648         +        0
     Intr   47783   47938         +        0
     Intr   48041   48196         +        0
     Term   48315   48388         +        0

>2827513        /19281

   len =    1222   nex =       2

     Init   48103   48196         +        0
     Term   48315   48388         +        0

>2827513        /3172

   len =     414   nex =       1

     Sngl   69669   70082         +        0

>2827513        /13678

   len =     696   nex =       0

>2827513        /9280
```

```
    len =      236    nex =        1

     Sngl   82731   82496          -         0

>2827538        /107860

    len =      756    nex =        2

     Init   10127   10535          +         0
     Term   10626   10882          +         0

>2827538        /35786

    len =     2746    nex =       10

     Init   11131   11383          +         0
     Intr   11755   11809          +         0
     Intr   11898   12053          +         0
     Intr   12143   12311          +         0
     Intr   12391   12455          +         0
     Intr   12534   12674          +         0
     Intr   12769   12885          +         0
     Intr   13024   13380          +         0
     Intr   13468   13525          +         0
     Term   13612   13876          +         0

>2827538        /22353

    len =     1781    nex =        1

     Sngl   22916   24696          +         0

>2827538        /41062

    len =     1775    nex =        3

     Init   30074   30181          +         0
     Intr   31148   31303          +         0
     Term   31408   31848          +         0

>2827538        /20948

    len =     2012    nex =        6

     Term   37215   36924          -         0
     Intr   37385   37302          -         0
     Intr   37555   37480          -         0
     Intr   37720   37640          -         0
     Intr   38064   37811          -         0
     Init   38618   38156          -         0

>2827538        /38360

    len =     2206    nex =        7

     Term   42538   42224          -         0
     Intr   42735   42673          -         0
     Intr   42925   42840          -         0
     Intr   43152   43023          -         0
    Intr   43349   43258          -        0
    Intr   43650   43437          -        0
    Init   44429   44185          -        0

>2827538         /39135

  len =     1970    nex =        5

    Term   48221   47882          -        0
    Intr   48414   48382          -        0
    Intr   48655   48508          -        0
    Intr   49319   48997          -        0
    Init   49851   49398          -        0

>2827538         /12041

  len =     1958    nex =        4

    Term   57306   56734          -        0
    Intr   57614   57483          -        0
    Intr   57766   57699          -        0
    Init   58691   58063          -        0

>2827538         /13832

  len =     1234    nex =        2

    Term   81460   80806          -        0
    Init   82039   81838          -        0

>2827538         /9746

  len =     2173    nex =        7

    Term   84745   84562          -        0
    Intr   84904   84822          -        0
    Intr   85251   84998          -        0
    Intr   85601   85349          -        0
    Intr   85852   85679          -        0
    Intr   86443   86358          -        0
    Init   86734   86552          -        0

>2827538         /18053

  len =     1301    nex =        1

    Sngl    9200    7900          -        0

>2827644         /40232

  len =     2013    nex =        7

    Init   50573   51055          +        0
    Intr   51150   51288          +        0
    Intr   51431   51539          +        0
    Intr   51732   51841          +        0
    Intr   51956   52103          +        0
    Intr   52222   52286          +        0
    Term   52373   52585          +        0
```

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```
>2827644        /15834

   len =    2350   nex =       3

    Term   59427   58305       -       0
    Intr   60290   60054       -       0
    Init   60647   60399       -       0

>2827644        /32637

   len =    2027   nex =       9

    Init   88415   88513       +       0
    Intr   88598   88726       +       0
    Intr   88827   88916       +       0
    Intr   89005   89155       +       0
    Intr   89262   89371       +       0
    Intr   89447   89518       +       0
    Intr   89612   89717       +       0
    Intr   89802   89996       +       0
    Term   90088   90441       +       0

>2827698        /29775

   len =    2250   nex =       6

    Init   21347   21716       +       0
    Intr   21801   21847       +       0
    Intr   21943   22057       +       0
    Intr   22752   22796       +       0
    Intr   23036   23131       +       0
    Term   23213   23596       +       0

>2827698        /17160

   len =    1210   nex =       1

    Sngl   24108   25313       +       0

>2827698        /36946

   len =    2069   nex =       5

    Init   28482   28521       +       0
    Intr   28627   28665       +       0
    Intr   28798   29172       +       0
    Intr   29262   29317       +       0
    Term   29444   29760       +       0

>2827698        /16111

   len =    1796   nex =       3

    Init    3992    4615       +       0
    Intr    4773    4960       +       0
    Term    5255    5787       +       0

>2828180        /31285

   len =    1117   nex =       3
```

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```
    Init   41115   41311         +        0
    Intr   41410   41571         +        0
    Term   41636   42231         +        0

>2828180       /9396

   len =     500   nex =        1

    Sngl   41747   42246         +        0

>2828180       /115921

   len =     392   nex =        1

    Sngl   41841   42232         +        0

>2828180       /32771

   len =    2958   nex =       12

    Init   53810   53931         +        0
    Intr   54276   54407         +        0
    Intr   54599   54657         +        0
    Intr   54939   55069         +        0
    Intr   55154   55239         +        0
    Intr   55348   55379         +        0
    Intr   55608   55769         +        0
    Intr   55869   55941         +        0
    Intr   56027   56127         +        0
    Intr   56276   56368         +        0
    Intr   56471   56517         +        0
    Term   56610   56767         +        0

>2828182       /5344

   len =    1193   nex =        2

    Init   11326   11889         +        0
    Term   11959   12518         +        0

>2828182       /35527

   len =    1242   nex =        2

    Init   12894   13457         +        0
    Term   13541   14135         +        0

>2828182       /207083

   len =    1835   nex =        3

    Term   60046   59494         -        0
    Intr   60847   60710         -        0
    Init   61328   61093         -        0

>2828182       /17482

   len =    1854   nex =        3
```

```
     Term   60046   59509        -        0
     Intr   60847   60710        -        0
     Init   61362   61093        -        0

>2828182         /158804

    len =      501    nex =        1

     Sngl   62845   62345        -        0

>2828182         /15560

    len =     2329    nex =        3

     Term   62937   62347        -        0
     Intr   63667   63187        -        0
     Init   64675   64169        -        0

>2828182         /17411

    len =     1728    nex =        2

     Init   79315   79968        +        0
     Term   80239   81042        +        0

>2828182         /13813

    len =     1618    nex =        4

     Init   81708   81785        +        0
     Intr   81894   82074        +        0
     Intr   82162   82259        +        0
     Term   82353   82976        +        0

>2828182         /22460

    len =      850    nex =        1

     Sngl   85041   85888        +        0

>2828183         /9655

    len =     1611    nex =        8

     Init   15617   15682        +        0
     Intr   15785   15864        +        0
     Intr   15960   16044        +        0
     Intr   16138   16185        +        0
     Intr   16274   16342        +        0
     Intr   16428   16583        +        0
     Intr   16725   16818        +        0
     Term   16920   17227        +        0

>2828183         /15495

    len =      610    nex =        1

     Sngl    2072    1467        -        0

>2828183         /38900
```

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```
     len =     2564    nex =       7

      Term   33533    33168         -         0
      Intr   33682    33616         -         0
      Intr   33903    33802         -         0
      Intr   34116    34003         -         0
      Intr   34399    34226         -         0
      Intr   35100    34669         -         0
      Init   35731    35192         -         0

>2828183        /18973

     len =      405    nex =       1

      Sngl   39229    38825         -         0

>2828184        /118036

     len =      658    nex =       2

      Init   14494    14634         +         0
      Term   14779    15151         +         0

>2828184        /11922

     len =     1978    nex =       6

      Init   42361    42487         +         0
      Intr   42866    42902         +         0
      Intr   43191    43413         +         0
      Intr   43509    43670         +         0
      Intr   43775    43897         +         0
      Term   43999    44338         +         0

>2828184        /17188

     len =     3468    nex =      12

      Init   54034    54226         +         0
      Intr   54348    54434         +         0
      Intr   54570    54675         +         0
      Intr   54848    54883         +         0
      Intr   55623    55674         +         0
      Intr   55958    56022         +         0
      Intr   56150    56206         +         0
      Intr   56372    56444         +         0
      Intr   56529    56615         +         0
      Intr   56712    56877         +         0
      Intr   56959    57068         +         0
      Term   57147    57481         +         0

>2828185        /102248

     len =     1092    nex =       2

      Init   13901    14405         +         0
      Term   14538    14992         +         0

>2828185        /14679
```

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```
    len =     501    nex =       1

     Sngl   19843    20343        +        0

>2828185        /33972

    len =    2385    nex =       9

     Init   53063    53348        +        0
     Intr   53686    53760        +        0
     Intr   53854    53955        +        0
     Intr   54029    54082        +        0
     Intr   54183    54275        +        0
     Intr   54389    54481        +        0
     Intr   54563    54667        +        0
     Intr   54744    54842        +        0
     Term   54920    55447        +        0

>2828185        /3988

    len =    1995    nex =       9

     Init   53214    53348        +        0
     Intr   53686    53760        +        0
     Intr   53854    53955        +        0
     Intr   54029    54082        +        0
     Intr   54183    54275        +        0
     Intr   54389    54481        +        0
     Intr   54536    54667        +        0
     Intr   54744    54842        +        0
     Term   54920    55208        +        0

>2828185        /15373

    len =    1398    nex =       6

     Init   68405    68507        +        0
     Intr   68622    68658        +        0
     Intr   68914    68999        +        0
     Intr   69103    69198        +        0
     Intr   69384    69546        +        0
     Term   69646    69802        +        0

>2828186        /108599

    len =    1481    nex =       4

     Init   14950    15033        +        0
     Intr   15143    15301        +        0
     Intr   15523    15749        +        0
     Term   15975    16194        +        0

>2828186        /20057

    len =    1042    nex =       2

     Term   42331    42168        -        0
     Init   43209    43006        -        0
```

```
>2828186        /116465

   len =     884    nex =        1

    Sngl   43436   44319          +         0

>2828186        /36363

   len =    1150    nex =        1

    Sngl   53511   53675          +         0

>2828186        /117347

   len =     791    nex =        1

    Sngl   60600   61390          +         0

>2828186        /6461

   len =     859    nex =        3

    Init   71764   72268          +         0
    Intr   72381   72437          +         0
    Term   72534   72612          +         0

>2828187        /23099

   len =    1114    nex =        2

    Term   64086   63766          -         0
    Init   64879   64636          -         0

>2828187        /9905

   len =    1351    nex =        6

    Term   72205   71968          -         0
    Intr   72362   72299          -         0
    Intr   72523   72492          -         0
    Intr   72708   72611          -         0
    Intr   73206   73071          -         0
    Init   73318   73233          -         0

>2828187        /91844

   len =    1425    nex =        5

    Term   72205   71961          -         0
    Intr   72362   72299          -         0
    Intr   72523   72492          -         0
    Intr   72708   72611          -         0
    Init   73206   73071          -         0

>2828187        /142899

   len =    1418    nex =        5

    Term   72205   71968          -         0
    Intr   72362   72299          -         0
```

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```
          Intr   72523   72492        -       0
          Intr   72708   72611        -       0
          Init   73206   73071        -       0

    >2828188       /17485

         len =     879   nex =       3

          Init   27707   27799        +       0
          Intr   27901   28043        +       0
          Term   28241   28522        +       0

    >2828188       /1983

         len =    2312   nex =      10

          Init   30424   30603        +       0
          Intr   30707   30804        +       0
          Intr   30896   30993        +       0
          Intr   31098   31147        +       0
          Intr   31561   31656        +       0
          Intr   31753   31842        +       0
          Intr   31914   32006        +       0
          Intr   32089   32184        +       0
          Intr   32288   32353        +       0
          Term   32425   32735        +       0

    >2828188       /2949

         len =     506   nex =       1

          Sngl   37555   37050        -       0

    >2828188       /34409

         len =    1630   nex =       2

          Init   49022   49121        +       0
          Term   49382   50625        +       0

    >2828188       /101070

         len =     672   nex =       1

          Sngl   68221   68889        +       0

    >2828278       /93971

         len =     630   nex =       1

          Sngl   20720   20517        -       0

    >2828278       /9641

         len =    1690   nex =       2

          Term   25562   25106        -       0
          Init   26162   26038        -       0

    >2828278       /3981
```

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```
len =     1341    nex =       3

  Term   28518    27857        -         0
  Intr   28679    28602        -         0
  Init   29197    28762        -         0

>2828278        /101094

len =      261    nex =       1

  Sngl   47484    47744        +         0

>2828278        /36576

len =     2509    nex =       7

  Term   57030    56730        -         0
  Intr   57325    57174        -         0
  Intr   57571    57416        -         0
  Intr   57973    57688        -         0
  Intr   58172    58056        -         0
  Intr   58336    58274        -         0
  Init   59238    58896        -         0

>2832611        /6321

len =     2846    nex =       2

  Init   12902    13264        +         0
  Term   14896    15747        +         0

>2832611        /115884

len =     2174    nex =       5

  Init   26411    26644        +         0
  Intr   26778    27002        +         0
  Intr   27714    27932        +         0
  Intr   28070    28131        +         0
  Term   28235    28584        +         0

>2832611        /8768

len =     1456    nex =       4

  Init   29030    29347        +         0
  Intr   29729    29811        +         0
  Intr   29893    30007        +         0
  Term   30105    30485        +         0

>2832611        /38712

len =     2988    nex =      12

  Init   35656    36292        +         0
  Intr   36401    36475        +         0
  Intr   36567    36662        +         0
  Intr   36750    36835        +         0
  Intr   36921    37041        +         0
```

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```
    Intr   37124   37203        +       0
    Intr   37298   37360        +       0
    Intr   37467   37573        +       0
    Intr   37704   37789        +       0
    Intr   37867   37988        +       0
    Intr   38095   38206        +       0
    Term   38306   38643        +       0

>2832611       /92096

   len =    1527   nex =       6

    Term   67967   67742        -       0
    Intr   68134   68059        -       0
    Intr   68294   68206        -       0
    Intr   68482   68429        -       0
    Intr   68719   68580        -       0
    Init   68873   68799        -       0

>2832611       /8996

   len =    1431   nex =       4

    Term   70354   70256        -       0
    Intr   70528   70448        -       0
    Intr   71169   70723        -       0
    Init   71686   71473        -       0

>2832611       /109513

   len =    1954   nex =       4

    Term   82219   81803        -       0
    Intr   82755   82537        -       0
    Intr   83404   83180        -       0
    Init   83756   83517        -       0

>2832639       /155370

   len =     809   nex =       4

    Init   13801   13917        +       0
    Intr   13999   14045        +       0
    Intr   14121   14217        +       0
    Term   14310   14609        +       0

>2832639       /16226

   len =    1821   nex =       0

>2832639       /10320

   len =     580   nex =       1

    Sngl   22887   22331        -       0

>2832639       /24883

   len =     490   nex =       1
```

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```
      Sngl   28760   29244         +       0

>2832639       /14794

     len =    1378   nex =       3

      Term   29423   29196         -       0
      Intr   29908   29796         -       0
      Init   30573   30411         -       0

>2832639       /907

     len =     802   nex =       1

      Sngl   41456   40655         -       0

>2832639       /10310

     len =    2754   nex =      15

      Init   52225   52432         +       0
      Intr   52507   52573         +       0
      Intr   52668   52761         +       0
      Intr   52853   52909         +       0
      Intr   53005   53074         +       0
      Intr   53159   53223         +       0
      Intr   53319   53400         +       0
      Intr   53494   53543         +       0
      Intr   53622   53765         +       0
      Intr   53843   53913         +       0
      Intr   53979   54048         +       0
      Intr   54133   54198         +       0
      Intr   54315   54473         +       0
      Intr   54555   54652         +       0
      Term   54755   54978         +       0

>2832639       /15817

     len =     753   nex =       1

      Sngl   61848   61096         -       0

>2832639       /7501

     len =    1667   nex =       3

      Term   62268   61093         -       0
      Intr   62493   62378         -       0
      Init   62759   62575         -       0

>2832639       /35285

     len =     850   nex =       4

      Init    8531    8838         +       0
      Intr    8913    9050         +       0
      Intr    9136    9198         +       0
      Term    9284    9373         +       0

>2832639       /31734
```

```
    len =    1358   nex =       4

     Term   94255   94004          -         0
     Intr   94442   94354          -         0
     Intr   94664   94560          -         0
     Init   94843   94774          -         0

>2832639       /24295

    len =     926   nex =       2

     Init   95837   96095          +         0
     Term   96192   96466          +         0

>2832639       /29009

    len =    1136   nex =       1

     Sngl   97830   98023          +         0

>2832667       /113536

    len =     701   nex =       2

     Init   14746   14852          +         0
     Term   14974   15446          +         0

>2832667       /26401

    len =     881   nex =       2

     Init   16072   16194          +         0
     Term   16286   16952          +         0

>2832667       /8156

    len =    1771   nex =       3

     Term   20352   19919          -         0
     Intr   20953   20899          -         0
     Init   21689   21342          -         0

>2832667       /19552

    len =    1788   nex =       3

     Term   20352   19913          -         0
     Intr   20953   20899          -         0
     Init   21700   21342          -         0

>2832667       /4390

    len =    2022   nex =       2

     Init   24444   24552          +         0
     Term   24642   25004          +         0

>2832667       /20563
```

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```
    len =      337    nex =         1

     Sngl   24653   24317           -         0

>2832667          /33086

    len =      578    nex =         1

     Sngl   27162   26726           -         0

>2832667          /111376

    len =     1337    nex =         3

     Term   27162   26726           -         0
     Intr   27443   27248           -         0
     Init   28062   27564           -         0

>2832667          /41007

    len =     1362    nex =         3

     Term   27162   26726           -         0
     Intr   27443   27248           -         0
     Init   28083   27564           -         0

>2832667          /6848

    len =     1157    nex =         1

     Sngl    3909    2753           -         0

>2832667          /5132

    len =     1474    nex =         5

     Init   52118   52255           +         0
     Intr   52656   52754           +         0
     Intr   52930   52982           +         0
     Intr   53135   53208           +         0
     Term   53464   53591           +         0

>2832667          /35323

    len =     1474    nex =         5

     Init   52120   52255           +         0
     Intr   52656   52754           +         0
     Intr   52930   52982           +         0
     Intr   53135   53208           +         0
     Term   53464   53593           +         0

>2832667          /4495

    len =     2114    nex =         1

     Sngl   59794   60466           +         0

>2832667          /2081
```

```
    len =    1232   nex =       3

     Init   68491   69116       +         0
     Intr   69422   69533       +         0
     Term   69623   69722       +         0

>2832667        /12272

    len =    2316   nex =       5

     Init   68540   69116       +         0
     Intr   69422   69533       +         0
     Intr   69623   69794       +         0
     Intr   69883   70085       +         0
     Term   70296   70411       +         0

>2832667        /2673

    len =     320   nex =       1

     Sngl   72959   72640       -         0

>2832689        /2075

    len =     595   nex =       1

     Sngl   42824   43418       +         0

>2832689        /5753

    len =     730   nex =       1

     Sngl   42881   43602       +         0

>2832689        /33965

    len =     680   nex =       1

     Sngl   42885   43564       +         0

>2832689        /34867

    len =    2058   nex =       6

     Init   65633   65767       +         0
     Intr   65857   66031       +         0
     Intr   66109   66211       +         0
     Intr   66295   66522       +         0
     Intr   66616   66834       +         0
     Term   66931   67577       +         0

>2832689        /26737

    len =    1390   nex =       2

     Init    6827    6884       +         0
     Term    6976    8196       +         0

>2833627        /38715
```

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```
    len =     4289    nex =        8

      Init   18099    18163          +         0
      Intr   18434    18574          +         0
      Intr   18921    18969          +         0
      Intr   19215    19331          +         0
      Intr   19412    19465          +         0
      Intr   19862    19950          +         0
      Intr   20099    20204          +         0
      Term   20282    20669          +         0

>2833627        /30652

    len =     2745    nex =        8

      Init   18099    18163          +         0
      Intr   18434    18574          +         0
      Intr   18921    18969          +         0
      Intr   19215    19331          +         0
      Intr   19412    19465          +         0
      Intr   19862    19950          +         0
      Intr   20099    20204          +         0
      Term   20282    20669          +         0

>2833627        /38934

    len =     2757    nex =        8

      Init   18099    18163          +         0
      Intr   18434    18574          +         0
      Intr   18921    18969          +         0
      Intr   19215    19331          +         0
      Intr   19412    19465          +         0
      Intr   19862    19950          +         0
      Intr   20099    20204          +         0
      Term   20282    20681          +         0

>2833627        /29043

    len =     2111    nex =        8

      Init   67181    67444          +         0
      Intr   67542    67618          +         0
      Intr   67706    67828          +         0
      Intr   68112    68216          +         0
      Intr   68331    68403          +         0
      Intr   68482    68749          +         0
      Intr   68837    68966          +         0
      Term   69054    69291          +         0

>2842474        /10704

    len =      825    nex =        3

      Init   21629    21886          +         0
      Intr   21961    21999          +         0
      Term   22252    22453          +         0

>2842474        /5117
```

```
     len =     734    nex =        1

       Sngl   23867   24227         +        0

>2842474        /1885

     len =    2068    nex =        7

       Init   35358   35949         +        0
       Intr   36030   36183         +        0
       Intr   36287   36350         +        0
       Intr   36434   36537         +        0
       Intr   36679   36748         +        0
       Intr   36860   36925         +        0
       Term   37010   37425         +        0

>2842474        /41008

     len =    1281    nex =        2

       Init   37554   37887         +        0
       Term   38343   38834         +        0

>2842474        /906

     len =    3084    nex =       16

       Term   39452   39140         -        0
       Intr   39647   39559         -        0
       Intr   39923   39818         -        0
       Intr   40163   40038         -        0
       Intr   40346   40261         -        0
       Intr   40503   40450         -        0
       Intr   40671   40608         -        0
       Intr   40868   40773         -        0
       Intr   41029   40955         -        0
       Intr   41151   41117         -        0
       Intr   41302   41233         -        0
       Intr   41481   41383         -        0
       Intr   41624   41597         -        0
       Intr   41822   41711         -        0
       Intr   42001   41911         -        0
       Init   42223   42084         -        0

>2842474        /40688

     len =    2028    nex =        5

       Term   62730   62375         -        0
       Intr   63029   62817         -        0
       Intr   63288   63118         -        0
       Intr   63523   63407         -        0
       Init   63946   63768         -        0

>2842474        /35774

     len =    4104    nex =        3

       Term   68866   68144         -        0
       Intr   70164   69899         -        0
     Init   72247   71953          -       0

>2842474        /18360

    len =     1882   nex =        6

     Term    7149    6711          -       0
     Intr    7371    7305          -       0
     Intr    7647    7537          -       0
     Intr    7915    7763          -       0
     Intr    8238    8171          -       0
     Init    8592    8366          -       0

>2853071        /19264

    len =     2650   nex =        3

     Init   28721   29386          +       0
     Intr   29475   30150          +       0
     Term   30940   31017          +       0

>2853071        /42503

    len =     1695   nex =        1

     Sngl    4329    2635          -       0

>2853071        /13295

    len =     1851   nex =        8

     Init   49706   49914          +       0
     Intr   50003   50111          +       0
     Intr   50216   50338          +       0
     Intr   50428   50525          +       0
     Intr   50616   50742          +       0
     Intr   50822   50907          +       0
     Intr   50994   51119          +       0
     Term   51220   51556          +       0

>2853071        /24087

    len =      799   nex =        1

     Sngl   74493   75291          +       0

>2853071        /143232

    len =      689   nex =        2

     Term   76634   76317          -       0
     Init   77005   76728          -       0

>2864607        /34819

    len =     1657   nex =        3

     Init   11411   11849          +       0
     Intr   12198   12373          +       0
     Term   12788   13067          +       0
```

```
>2864607       /31896

   len =     490   nex =       1

    Sngl   35491   35976        +        0

>2864607       /20798

   len =    1016   nex =       2

    Init   50921   51566        +        0
    Term   51635   51936        +        0

>2864607       /30539

   len =     995   nex =       2

    Init   50974   51566        +        0
    Term   51635   51968        +        0

>2864607       /30310

   len =     779   nex =       3

    Term   56737   56505        -        0
    Intr   56871   56822        -        0
    Init   57157   56983        -        0

>2864607       /29964

   len =     877   nex =       4

    Term   56737   56470        -        0
    Intr   56871   56822        -        0
    Intr   57157   56983        -        0
    Init   57346   57251        -        0

>2864607       /115583

   len =    1150   nex =       1

    Sngl   91469   90322        -        0

>2880038       /39751

   len =    2230   nex =       7

    Term   33273   33017        -        0
    Intr   33540   33439        -        0
    Intr   33821   33808        -        0
    Intr   34285   34177        -        0
    Intr   34463   34390        -        0
    Intr   34593   34550        -        0
    Init   34847   34762        -        0

>2880038       /20095

   len =    2306   nex =       8
```

```
        Term   33273   32976          -        0
        Intr   33540   33439          -        0
        Intr   33821   33808          -        0
        Intr   34285   34177          -        0
        Intr   34463   34390          -        0
        Intr   34593   34550          -        0
        Intr   34847   34762          -        0
        Init   35271   35063          -        0

>2880038        /31304

      len =      310    nex =        1

        Sngl      75     382          +        0

>2894557        /98545

      len =      953    nex =        2

        Term   11319   10903          -        0
        Init   11855   11346          -        0

>2894557        /14645

      len =     1007    nex =        3

        Term   11187   10877          -        0
        Intr   11502   11346          -        0
        Init   11883   11602          -        0

>2894557        /14462

      len =     1180    nex =        5

        Term   13853   13556          -        0
        Intr   14042   14002          -        0
        Intr   14172   14109          -        0
        Intr   14432   14276          -        0
        Init   14735   14527          -        0

>2894557        /24573

      len =     1059    nex =        3

        Term   17997   17644          -        0
        Intr   18258   18102          -        0
        Init   18702   18484          -        0

>2894557        /16643

      len =     1411    nex =        3

        Term   26860   26567          -        0
        Intr   27098   26942          -        0
        Init   27977   27679          -        0

>2894557        /1004

      len =     1369    nex =        3
```

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```
        Init   28664   28756          +        0
        Intr   29030   29218          +        0
        Term   29365   29739          +        0

>2894557        /4764

      len =     610   nex =       1

        Sngl   30727   30125          -        0

>2894557        /23223

      len =     670   nex =       2

        Term   30491   30129          -        0
        Init   30795   30617          -        0

>2894557        /28411

      len =    1931   nex =       6

        Term    1991    1564          -        0
        Intr    2137    2084          -        0
        Intr    2311    2228          -        0
        Intr    2623    2409          -        0
        Intr    3127    3028          -        0
        Init    3494    3369          -        0

>2894557        /17340

      len =    5636   nex =       8

        Term   52206   51907          -        0
        Intr   52347   52286          -        0
        Intr   52539   52453          -        0
        Intr   52749   52631          -        0
        Intr   53083   52845          -        0
        Intr   53233   53177          -        0
        Intr   54012   53381          -        0
        Init   57542   57102          -        0

>2894557        /32972

      len =     328   nex =       1

        Sngl   73075   72748          -        0

>2894557        /33172

      len =    2020   nex =       4

        Term   73486   72801          -        0
        Intr   74137   73972          -        0
        Intr   74427   74239          -        0
        Init   74820   74549          -        0

>2894557        /20758

      len =    1959   nex =       4
```

```
     Term   73486   72863          -        0
     Intr   74137   73972          -        0
     Intr   74427   74239          -        0
     Init   74821   74549          -        0

>2894591       /37898

   len =     1361   nex =        7

     Init       4      48          +        0
     Intr     125     263          +        0
     Intr     346     421          +        0
     Intr     502     624          +        0
     Intr     715     868          +        0
     Intr     975    1008          +        0
     Term    1090    1361          +        0

>2894591       /4613

   len =      818   nex =        1

     Sngl   11818   12635          +        0

>2894591       /12013

   len =     2086   nex =        3

     Init   29017   29255          +        0
     Intr   29840   30037          +        0
     Term   30126   30344          +        0

>2894591       /38412

   len =     2218   nex =       10

     Term    1518    1201          -        0
     Intr    1688    1600          -        0
     Intr    1839    1768          -        0
     Intr    2024    1922          -        0
     Intr    2182    2114          -        0
     Intr    2336    2274          -        0
     Intr    2511    2416          -        0
     Intr    2749    2598          -        0
     Intr    3311    2863          -        0
     Init    3418    3345          -        0

>2894591       /33385

   len =     1224   nex =        3

     Init   48888   49463          +        0
     Intr   49539   49711          +        0
     Term   49804   50111          +        0

>2894591       /13507

   len =     1610   nex =        2

     Init   53993   54524          +        0
     Term   55134   55602          +        0
```

```
>2914688        /27602

   len =     827    nex =        2

    Init   32845   33192         +        0
    Term   33282   33671         +        0

>2914688        /27570

   len =     500    nex =        1

    Sngl    3483    3969         +        0

>2914688        /30472

   len =     650    nex =        2

    Term   43833   43650         -        0
    Init   44299   43939         -        0

>2914688        /39917

   len =    1453    nex =        2

    Term   63880   62989         -        0
    Init   64441   63995         -        0

>2914688        /7865

   len =     398    nex =        1

    Sngl   74566   74860         +        0

>2914688        /154660

   len =     406    nex =        1

    Sngl   75023   75428         +        0

>2914688        /3956

   len =     380    nex =        1

    Sngl   75051   75430         +        0

>2914688        /146794

   len =     202    nex =        1

    Sngl   75123   75324         +        0

>2914688        /23742

   len =     987    nex =        1

    Sngl   87641   87085         -        0

>2914688        /28031
```

```
     len =     1091    nex =        1

      Sngl   87641   87085          -         0

>2924505        /21896

     len =     2830    nex =        9

      Term   21014   20638          -         0
      Intr   21499   21397          -         0
      Intr   21723   21644          -         0
      Intr   21889   21807          -         0
      Intr   22051   22003          -         0
      Intr   22205   22140          -         0
      Intr   22801   22752          -         0
      Intr   23090   22966          -         0
      Init   23467   23242          -         0

>2924505        /19273

     len =     1736    nex =        7

      Init   28770   29087          +         0
      Intr   29392   29483          +         0
      Intr   29564   29673          +         0
      Intr   29776   29839          +         0
      Intr   29938   30002          +         0
      Intr   30084   30149          +         0
      Term   30249   30505          +         0

>2924505        /265

     len =      850    nex =        2

      Term   42073   41536          -         0
      Init   42383   42158          -         0

>2924505        /15093

     len =     1849    nex =        2

      Init   54147   54502          +         0
      Term   55034   55995          +         0

>2924505        /39154

     len =     1631    nex =        4

      Init   64280   64770          +         0
      Intr   64863   65158          +         0
      Intr   65418   65558          +         0
      Term   65652   65910          +         0

>2924505        /7108

     len =     1477    nex =        4

      Init   64436   64770          +         0
      Intr   64863   65158          +         0
      Intr   65418   65558          +         0
```

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```
     Term   65652   65912        +       0

>2924651       /116144

    len =     550   nex =       1

     Sngl   11422   11968        +       0

>2924651       /4228

    len =    1336   nex =       1

     Sngl   16008   16108        +       0

>2924651       /41343

    len =    1397   nex =       2

     Init   16012   16108        +       0
     Term   16483   17408        +       0

>2924651       /37811

    len =    3460   nex =      14

     Term   17854   17679        -       0
     Intr   17949   17883        -       0
     Intr   18132   18040        -       0
     Intr   18319   18226        -       0
     Intr   18472   18400        -       0
     Intr   19189   19124        -       0
     Intr   19363   19286        -       0
     Intr   19517   19431        -       0
     Intr   20061   19866        -       0
     Intr   20202   20146        -       0
     Intr   20333   20276        -       0
     Intr   20770   20446        -       0
     Intr   20948   20865        -       0
     Init   21138   21036        -       0

>2924651       /11706

    len =     797   nex =       4

     Init   27009   27125        +       0
     Intr   27212   27285        +       0
     Intr   27349   27473        +       0
     Term   27545   27805        +       0

>2924651       /101608

    len =     798   nex =       3

     Init   27010   27125        +       0
     Intr   27212   27473        +       0
     Term   27545   27807        +       0

>2924651       /11215

    len =    1990   nex =       4
```

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```
     Term   40287   39899        -        0
     Intr   40599   40363        -        0
     Intr   41110   40743        -        0
     Init   41882   41190        -        0

>2924651       /124621

   len =    1394   nex =       5

     Init   42248   42476        +        0
     Intr   42705   42841        +        0
     Intr   42918   42975        +        0
     Intr   43069   43146        +        0
     Term   43242   43641        +        0

>2924651       /115662

   len =    1586   nex =       3

     Term   54089   53651        -        0
     Intr   54599   54185        -        0
     Init   55236   54985        -        0

>2924651       /24140

   len =     989   nex =       5

     Init   64216   64414        +        0
     Intr   64498   64577        +        0
     Intr   64656   64726        +        0
     Intr   64819   64872        +        0
     Term   64968   65204        +        0

>2924651       /41440

   len =     520   nex =       2

     Term   67750   67536        -        0
     Init   68055   67838        -        0

>2924651       /27491

   len =    1390   nex =       7

     Term   68218   68177        -        0
     Intr   68390   68313        -        0
     Intr   68583   68492        -        0
     Intr   68745   68667        -        0
     Intr   68963   68830        -        0
     Intr   69280   69052        -        0
     Init   69566   69388        -        0

>2924651       /18901

   len =    2963   nex =      12

     Term   73319   73016        -        0
     Intr   73588   73415        -        0
     Intr   73756   73694        -        0
     Intr   73960   73853         -         0
     Intr   74160   74045         -         0
     Intr   74372   74246         -         0
     Intr   74635   74466         -         0
     Intr   74806   74728         -         0
     Intr   75007   74896         -         0
     Intr   75150   75062         -         0
     Intr   75362   75232         -         0
     Init   75978   75624         -         0

>2924651        /111348

    len =     681   nex =        2

     Init   79079   79259         +         0
     Term   79349   79759         +         0

>2924652        /4911

    len =    1030   nex =        3

     Term   19435   19201         -         0
     Intr   19824   19635         -         0
     Init   20222   20090         -         0

>2924652        /36891

    len =    1540   nex =        3

     Term   34140   33486         -         0
     Intr   34498   34229         -         0
     Init   35025   34629         -         0

>2924652        /6935

    len =    1820   nex =        3

     Term   34140   33312         -         0
     Intr   34498   34229         -         0
     Init   35131   34629         -         0

>2924653        /24054

    len =     985   nex =        1

     Sngl   18725   19123         +         0

>2924653        /40303

    len =    2207   nex =        7

     Init   48858   49136         +         0
     Intr   49281   49579         +         0
     Intr   49695   49920         +         0
     Intr   50013   50258         +         0
     Intr   50352   50462         +         0
     Intr   50538   50669         +         0
     Term   50749   51064         +         0

>2924653        /37280
```

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```
     len =     2358    nex =       10

      Init     5790     5865          +         0
      Intr     6335     6391          +         0
      Intr     6473     6632          +         0
      Intr     6769     6851          +         0
      Intr     6950     7009          +         0
      Intr     7106     7214          +         0
      Intr     7290     7342          +         0
      Intr     7437     7517          +         0
      Intr     7595     7707          +         0
      Term     7811     8147          +         0

>2924653        /10022

     len =     2002    nex =        4

      Init    64053    64145          +         0
      Intr    64317    64444          +         0
      Intr    64584    64688          +         0
      Term    65141    65503          +         0

>2924653        /12547

     len =     2003    nex =        4

      Init    64053    64145          +         0
      Intr    64317    64444          +         0
      Intr    64584    64688          +         0
      Term    65141    65504          +         0

>2924653        /6841

     len =     1345    nex =        3

      Init    70235    70473          +         0
      Intr    70569    70632          +         0
      Term    71064    71579          +         0

>2924653        /22448

     len =     1249    nex =        3

      Init    70377    70473          +         0
      Intr    70569    70632          +         0
      Term    71064    71625          +         0

>2924653        /92808

     len =      850    nex =        3

      Term    74646    74511          -         0
      Intr    74845    74760          -         0
      Init    75351    74975          -         0

>2924654        /30054

     len =     2574    nex =        7
     Init    14617    14705          +         0
     Intr    15035    15246          +         0
     Intr    15355    15634          +         0
     Intr    15717    15788          +         0
     Intr    15879    15995          +         0
     Intr    16116    16181          +         0
     Term    16657    16957          +         0

>2924654         /37363

    len =     2618    nex =        6

     Init    14617    15246          +         0
     Intr    15355    15634          +         0
     Intr    15717    15788          +         0
     Intr    15879    15995          +         0
     Intr    16116    16181          +         0
     Term    16657    17013          +         0

>2924654         /41320

    len =     2533    nex =        7

     Init    14422    14705          +         0
     Intr    15035    15246          +         0
     Intr    15355    15634          +         0
     Intr    15717    15788          +         0
     Intr    15879    15995          +         0
     Intr    16116    16181          +         0
     Term    16657    16954          +         0

>2924654         /105970

    len =      997    nex =        1

     Sngl    29862    28866          -         0

>2924654         /37399

    len =     2110    nex =        5

     Init    41781    42184          +         0
     Intr    42289    42366          +         0
     Intr    42453    42522          +         0
     Intr    42650    42927          +         0
     Term    43021    43108          +         0

>2924655         /33830

    len =     2316    nex =       10

     Term    18451    18193          -         0
     Intr    18825    18612          -         0
     Intr    19016    18919          -         0
     Intr    19270    19142          -         0
     Intr    19331    19289          -         0
     Intr    19690    19580          -         0
     Intr    19925    19779          -         0
     Intr    20137    20024          -         0
     Intr    20329    20200          -         0
```

```
Init   20508   20379         -        0

>2924655       /37918

len =     799   nex =       3

 Init   34127   34257        +        0
 Intr   34452   34520        +        0
 Term   34618   34925        +        0

>2924728       /112173

len =     321   nex =       1

 Sngl   16725   16405        -        0

>2924728       /20919

len =    3224   nex =      12

 Init   39629   39916        +        0
 Intr   40038   40180        +        0
 Intr   40259   40351        +        0
 Intr   40438   40554        +        0
 Intr   40669   40764        +        0
 Intr   40904   41017        +        0
 Intr   41217   41270        +        0
 Intr   41480   41527        +        0
 Intr   41825   41975        +        0
 Intr   42080   42245        +        0
 Intr   42327   42396        +        0
 Term   42476   42852        +        0

>2924728       /15599

len =     290   nex =       1

 Sngl   46957   46668        -        0

>2924728       /32450

len =    2230   nex =       2

 Term   47403   46673        -        0
 Init   48900   48211        -        0

>2924728       /11304

len =    1048   nex =       1

 Sngl    4497    3866        -        0

>2924728       /11830

len =     740   nex =       3

 Term   56509   56324        -        0
 Intr   56849   56595        -        0
 Init   57063   56929        -        0
```

```
>2924729        /32977

    len =     597    nex =       1

      Sngl   32483   31887        -        0

>2924729        /8150

    len =    1108    nex =       3

      Term   37394   37188        -        0
      Intr   37888   37601        -        0
      Init   38295   37976        -        0

>2924729        /108595

    len =     698    nex =       1

      Sngl   41180   40483        -        0

>2924729        /27221

    len =    1539    nex =       3

      Term   60702   60586        -        0
      Intr   61397   61275        -        0
      Init   61782   61669        -        0

>2924729        /12817

    len =    1994    nex =       3

      Term   60702   60586        -        0
      Intr   61397   61275        -        0
      Init   62241   61669        -        0

>2924730        /14419

    len =     559    nex =       1

      Sngl    3605    3059        -        0

>2924730        /9214

    len =     328    nex =       1

      Sngl    8331    8658        +        0

>2924731        /34161

    len =    2210    nex =       8

      Init    3351    3568        +        0
      Intr    3663    3747        +        0
      Intr    3839    3906        +        0
      Intr    4037    4087        +        0
      Intr    4172    4245        +        0
      Intr    4652    4710        +        0
      Intr    4794    4870        +        0
      Term    5021    5301        +        0
```

```
>2924732        /23726

   len =     2127   nex =        6

    Term   15136   14749          -        0
    Intr   15419   15211          -        0
    Intr   15598   15512          -        0
    Intr   15788   15678          -        0
    Intr   16145   16078          -        0
    Init   16299   16228          -        0

>2924732        /118484

   len =     1335   nex =        2

    Term   17876   17178          -        0
    Init   18512   18040          -        0

>2924732        /110980

   len =     1726   nex =        6

    Term    2588    2145          -        0
    Intr    2744    2665          -        0
    Intr    2992    2845          -        0
    Intr    3155    3095          -        0
    Intr    3489    3265          -        0
    Init    3870    3763          -        0

>2924732        /10411

   len =      770   nex =        1

    Sngl   43741   42972          -        0

>2924732        /19177

   len =      829   nex =        1

    Sngl   45210   44382          -        0

>2924732        /37398

   len =     2350   nex =        9

    Term   72077   71790          -        0
    Intr   72317   72183          -        0
    Intr   72514   72395          -        0
    Intr   72722   72612          -        0
    Intr   73197   72982          -        0
    Intr   73331   73278          -        0
    Intr   73501   73430          -        0
    Intr   73771   73595          -        0
    Init   74137   73854          -        0

>2924733        /12093

   len =      573   nex =        2
```

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```
    Term   12077   11784          -        0
    Init   12356   12172          -        0

>2924733         /30414

  len =     397    nex =        2

    Init    2448    2519          +        0
    Term    2615    2844          +        0

>2924733         /147492

  len =     621    nex =        1

    Sngl   38573   37953          -        0

>2924733         /8989

  len =    2384    nex =        8

    Init     465     737          +        0
    Intr     820     941          +        0
    Intr    1362    1477          +        0
    Intr    1616    1689          +        0
    Intr    1800    2064          +        0
    Intr    2148    2279          +        0
    Intr    2384    2519          +        0
    Term    2615    2848          +        0

>2924733         /20896

  len =    2392    nex =        8

    Init     465     737          +        0
    Intr     820     941          +        0
    Intr    1362    1477          +        0
    Intr    1616    1689          +        0
    Intr    1800    2064          +        0
    Intr    2148    2279          +        0
    Intr    2384    2519          +        0
    Term    2615    2856          +        0

>2924733         /25282

  len =    2410    nex =        8

    Init     465     737          +        0
    Intr     820     941          +        0
    Intr    1362    1477          +        0
    Intr    1616    1689          +        0
    Intr    1800    2064          +        0
    Intr    2148    2279          +        0
    Intr    2384    2519          +        0
    Term    2615    2868          +        0

>2924733         /15239

  len =     976    nex =        1

    Sngl   47202   46227          -        0
```

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```
>2924733        /37419

   len =     1100    nex =        1

    Sngl    51162    52261         +        0

>2924733        /39485

   len =     2050    nex =        6

    Term    57509    57170         -        0
    Intr    57700    57603         -        0
    Intr    57992    57801         -        0
    Intr    58319    58246         -        0
    Intr    58474    58387         -        0
    Init    59210    58968         -        0

>2924733        /28716

   len =     2215    nex =        3

    Init     9164     9224         +        0
    Intr     9724    10187         +        0
    Term    10288    11378         +        0

>2924768        /954

   len =     1171    nex =        3

    Term    20675    20418         -        0
    Intr    20828    20757         -        0
    Init    21588    21272         -        0

>2924768        /22220

   len =      970    nex =        2

    Term    33367    33014         -        0
    Init    33981    33703         -        0

>2924768        /36488

   len =     3370    nex =       14

    Init    49401    49566         +        0
    Intr    49979    50055         +        0
    Intr    50164    50248         +        0
    Intr    50331    50457         +        0
    Intr    50547    50645         +        0
    Intr    50719    50786         +        0
    Intr    50867    50974         +        0
    Intr    51064    51141         +        0
    Intr    51259    51391         +        0
    Intr    51486    51582         +        0
    Intr    51750    51873         +        0
    Intr    51966    52074         +        0
    Intr    52362    52445         +        0
    Term    52537    52762         +        0
```

```
>2924768       /2443

    len =    2009   nex =       5

      Init   54917   55312        +        0
      Intr   55789   55930        +        0
      Intr   56024   56144        +        0
      Intr   56337   56399        +        0
      Term   56526   56925        +        0

>2924768       /40107

    len =    1793   nex =       2

      Init   55097   55930        +        0
      Term   56024   56889        +        0

>2947056       /35441

    len =    1884   nex =       7

      Term   24625   24082        -        0
      Intr   24833   24712        -        0
      Intr   25012   24909        -        0
      Intr   25146   25116        -        0
      Intr   25374   25244        -        0
      Intr   25569   25472        -        0
      Init   25953   25760        -        0

>2947056       /18146

    len =     884   nex =       5

      Term   52485   52355        -        0
      Intr   52728   52640        -        0
      Intr   52902   52813        -        0
      Intr   53019   52994        -        0
      Init   53238   53111        -        0

>2961335       /19666

    len =     970   nex =       3

      Init  100334  100454        +        0
      Intr  100557  100713        +        0
      Term  100813  101289        +        0

>2961335       /42504

    len =    1641   nex =       4

      Term  101428  101392        -        0
      Intr  102385  102247        -        0
      Intr  102589  102521        -        0
      Init  103032  102688        -        0

>2961335       /37217

    len =    1635   nex =       4
```

```
     Term  101428  101398          -         0
     Intr  102385  102247          -         0
     Intr  102589  102521          -         0
     Init  103032  102688          -         0

>2961335       /39963

   len =     889   nex =        1

     Sngl   31532   30644          -         0

>2961335       /36866

   len =    2013   nex =        4

     Term   31944   30671          -         0
     Intr   32165   32030          -         0
     Intr   32386   32252          -         0
     Init   32683   32529          -         0

>2961335       /21244

   len =    1212   nex =        1

     Sngl   33841   33295          -         0

>2961335       /322

   len =    2495   nex =        9

     Term   85172   85058          -         0
     Intr   85456   85295          -         0
     Intr   85703   85608          -         0
     Intr   85906   85842          -         0
     Intr   86542   86467          -         0
     Intr   86714   86632          -         0
     Intr   87177   86849          -         0
     Intr   87304   87258          -         0
     Init   87546   87386          -         0

>2961370       /14792

   len =    1218   nex =        2

     Init    2148    2639          +         0
     Term    2907    3365          +         0

>2961370       /41090

   len =     271   nex =        1

     Sngl    3095    3365          +         0

>2961370       /36110

   len =    2782   nex =       12

     Init   31733   31968          +         0
     Intr   32075   32200          +         0
     Intr   32291   32450          +         0
```

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```
        Intr   32544   32729         +       0
        Intr   32831   32947         +       0
        Intr   33047   33100         +       0
        Intr   33180   33251         +       0
        Intr   33395   33579         +       0
        Intr   33685   33779         +       0
        Intr   33862   33936         +       0
        Intr   34061   34144         +       0
        Term   34259   34514         +       0

>2961370       /9402

      len =      329   nex =        1

        Sngl   52923   53251         +       0

>2961370       /12881

      len =      375   nex =        1

        Sngl   88516   88142         -       0

>2979540       /12830

      len =     1150   nex =        2

        Term   31157   30569         -       0
        Init   31718   31304         -       0

>2979540       /11908

      len =      238   nex =        1

        Sngl   43116   43353         +       0

>2979540       /9990

      len =     1645   nex =        4

        Term   53451   53377         -       0
        Intr   53944   53849         -       0
        Intr   54297   54187         -       0
        Init   54719   54557         -       0

>2979540       /5467

      len =     3267   nex =        7

        Term   61277   60884         -       0
        Intr   61544   61503         -       0
        Intr   61708   61667         -       0
        Intr   61911   61812         -       0
        Intr   62080   62019         -       0
        Intr   62353   62272         -       0
        Init   63560   63356         -       0

>2979540       /100354

      len =     1123   nex =        3
```

```
         Term    68542    68068          -          0
         Intr    68676    68629          -          0
         Init    69047    68997          -          0

     >2979540         /18662

       len =     1011    nex =         3

         Init    84864    84965          +          0
         Intr    85100    85128          +          0
         Term    85223    85515          +          0

     >2979540         /9848

       len =      478    nex =         3

         Init    84863    84965          +          0
         Intr    85100    85128          +          0
         Term    85223    85340          +          0

     >2979540         /123760

       len =      631    nex =         3

         Term     9120     9002          -          0
         Intr     9356     9289          -          0
         Init     9632     9459          -          0

     >2979540         /35578

       len =      976    nex =         1

         Sngl     9822    10797          +          0

     >2980757         /96478

       len =      628    nex =         2

         Term   101920   101603          -          0
         Init   102230   102001          -          0

     >2980757         /29888

       len =     1706    nex =         2

         Init   105420   106491          +          0
         Term   106578   106835          +          0

     >2980757         /20759

       len =     1674    nex =         1

         Sngl   111308   109635          -          0

     >2980757         /32640

       len =     1975    nex =         5

         Term   113953   113573          -          0
         Intr   114107   114042          -          0
```

```
    Intr  114352  114249         -        0
    Intr  114505  114432         -        0
    Init  115547  115405         -        0

>2980757       /14193

   len =    2050   nex =       4

    Term  113953  113573         -        0
    Intr  114107  114042         -        0
    Intr  114352  114249         -        0
    Init  114505  114432         -        0

>2980757       /39459

   len =    2077   nex =       4

    Term  113953  113570         -        0
    Intr  114107  114042         -        0
    Intr  114352  114249         -        0
    Init  114505  114432         -        0

>2980757       /20888

   len =    3039   nex =       9

    Term  116278  115618         -        0
    Intr  116459  116365         -        0
    Intr  116906  116773         -        0
    Intr  117244  116981         -        0
    Intr  117420  117335         -        0
    Intr  117593  117501         -        0
    Intr  117776  117681         -        0
    Intr  117973  117866         -        0
    Init  118656  118366         -        0

>2980757       /17828

   len =    1390   nex =       3

    Term   19947   19431         -        0
    Intr   20153   20130         -        0
    Init   20797   20451         -        0

>2980757       /37878

   len =    1891   nex =       7

    Term   22249   22095         -        0
    Intr   22401   22353         -        0
    Intr   22603   22491         -        0
    Intr   22808   22697         -        0
    Intr   23041   22956         -        0
    Intr   23548   23465         -        0
    Init   23985   23709         -        0

>2980757       /40278

   len =    2700   nex =      10
```

```
        Init   62319   62472         +        0
        Intr   62599   62662         +        0
        Intr   62935   63182         +        0
        Intr   63261   63404         +        0
        Intr   63478   63629         +        0
        Intr   63712   63834         +        0
        Intr   63933   64110         +        0
        Intr   64204   64322         +        0
        Intr   64409   64523         +        0
        Term   64665   65018         +        0

>2980757       /7876

      len =     1620   nex =        5

        Init   70442   70911         +        0
        Intr   70996   71076         +        0
        Intr   71232   71333         +        0
        Intr   71626   71700         +        0
        Term   71818   72061         +        0

>2980757       /31287

      len =     2424   nex =        7

        Init    8024    8864         +        0
        Intr    9004    9089         +        0
        Intr    9190    9363         +        0
        Intr    9500    9614         +        0
        Intr    9732    9829         +        0
        Intr    9974   10109         +        0
        Term   10228   10447         +        0

>2980787       /27462

      len =     2028   nex =        4

        Term   46006   45949         -        0
        Intr   46321   46080         -        0
        Intr   46522   46410         -        0
        Init   47415   47305         -        0

>3004543       /39073

      len =     1651   nex =        5

        Init   15971   16170         +        0
        Intr   16291   16381         +        0
        Intr   16472   17062         +        0
        Intr   17134   17233         +        0
        Term   17529   17621         +        0

>3004543       /15729

      len =     1699   nex =        5

        Init   15980   16150         +        0
        Intr   16291   16381         +        0
        Intr   16472   17062         +        0
        Intr   17134   17233         +        0
```

```
     Term   17529   17678         +        0

>3004543        /1314

    len =     1330   nex =        5

     Init   21535   21572         +        0
     Intr   21645   21756         +        0
     Intr   21837   21934         +        0
     Intr   22311   22407         +        0
     Term   22482   22571         +        0

>3004543        /3350

    len =     1450   nex =        3

     Term   44799   44453         -        0
     Intr   45325   45228         -        0
     Init   45894   45618         -        0

>3004543        /36487

    len =      770   nex =        2

     Term   45325   45258         -        0
     Init   46027   45618         -        0

>3004543        /16223

    len =     1140   nex =        4

     Init   78104   78275         +        0
     Intr   78360   78542         +        0
     Intr   78629   78810         +        0
     Term   78902   79243         +        0

>3021263        /1667

    len =      868   nex =        1

     Sngl   23858   22991         -        0

>3033373        /21342

    len =      747   nex =        1

     Sngl   15077   15823         +        0

>3033373        /29605

    len =      595   nex =        1

     Sngl   39454   40045         +        0

>3033373        /6606

    len =     1090   nex =        1

     Sngl   79964   78877         -        0
```

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```
>3033373        /24400

   len =     2003    nex =        6

    Init    82999    83318          +         0
    Intr    83637    83702          +         0
    Intr    83796    83846          +         0
    Intr    83938    84017          +         0
    Intr    84308    84352          +         0
    Term    84467    85001          +         0

>3033373        /5509

   len =     3955    nex =       15

    Init    85397    85674          +         0
    Intr    85852    85906          +         0
    Intr    85992    86143          +         0
    Intr    86819    86858          +         0
    Intr    87036    87128          +         0
    Intr    87213    87328          +         0
    Intr    87417    87491          +         0
    Intr    87611    87650          +         0
    Intr    87739    87821          +         0
    Intr    87911    87988          +         0
    Intr    88122    88265          +         0
    Intr    88458    88525          +         0
    Intr    88608    88722          +         0
    Intr    88830    88986          +         0
    Term    89057    89351          +         0

>3036791        /34369

   len =     1935    nex =        7

    Term    30641    30380          -         0
    Intr    30794    30729          -         0
    Intr    30936    30872          -         0
    Intr    31426    31363          -         0
    Intr    31610    31501          -         0
    Intr    31784    31697          -         0
    Init    32314    31905          -         0

>3036791        /11954

   len =     1033    nex =        1

    Sngl    56760    55728          -         0

>3036791        /103458

   len =     1150    nex =        1

    Sngl    58836    59073          +         0

>3036791        /4329

   len =     1227    nex =        2

    Init    90374    91016          +         0
```

```
     Term   91266   91600         +       0

>3036791        /14578

   len =      880   nex =       1

     Sngl   96436   97315         +       0

>3046847        /8184

   len =     3010   nex =      10

     Term    8682    8425         -       0
     Intr    8887    8808         -       0
     Intr    9087    8960         -       0
     Intr    9281    9216         -       0
     Intr    9673    9622         -       0
     Intr    9870    9785         -       0
     Intr   10314   10151         -       0
     Intr   10511   10395         -       0
     Intr   10668   10598         -       0
     Init   11428   11126         -       0

>3046847        /37197

   len =     1990   nex =       5

     Term   11964   11553         -       0
     Intr   12305   12048         -       0
     Intr   12692   12466         -       0
     Intr   12917   12776         -       0
     Init   13535   13263         -       0

>3046847        /19893

   len =      858   nex =       1

     Sngl   39899   39042         -       0

>3046847        /92102

   len =      992   nex =       1

     Sngl   42005   41014         -       0

>3046847        /2037

   len =      585   nex =       2

     Term   43375   42957         -       0
     Init   43541   43466         -       0

>3046847        /95135

   len =     1247   nex =       2

     Init   49610   50038         +       0
     Term   50471   50856         +       0

>3046847        /9324
```

```
    len =     479    nex =        1

     Sngl   54499   54021          -         0

>3046849        /14126

    len =    1577    nex =        2

     Term   28105   27242          -         0
     Init   28795   28556          -         0

>3046849        /156665

    len =     839    nex =        2

     Term   28105   27980          -         0
     Init   28795   28556          -         0

>3046849        /3963

    len =    1488    nex =        2

     Term   39835   39089          -         0
     Init   40576   40199          -         0

>3046849        /13314

    len =    1665    nex =        5

     Init   44226   44488          +         0
     Intr   44558   44738          +         0
     Intr   44824   44990          +         0
     Intr   45085   45167          +         0
     Term   45367   45890          +         0

>3046849        /40824

    len =    1361    nex =        5

     Init   44387   44488          +         0
     Intr   44558   44738          +         0
     Intr   44824   44990          +         0
     Intr   45085   45167          +         0
     Term   45367   45747          +         0

>3046850        /32178

    len =    1227    nex =        5

     Term      49       1          -         0
     Intr     218     144          -         0
     Intr     570     392          -         0
     Intr     748     681          -         0
     Init    1227     972          -         0

>3046850        /23581

    len =    1553    nex =        4
```

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```
      Term   26495   26176          -        0
      Intr   27046   26959          -        0
      Intr   27255   27139          -        0
      Init   27728   27452          -        0

>3046850        /4275

     len =     1570   nex =        4

      Term   26495   26189          -        0
      Intr   27046   26959          -        0
      Intr   27255   27139          -        0
      Init   27752   27452          -        0

>3046850        /18867

     len =     2066   nex =        6

      Term   35882   35690          -        0
      Intr   36071   35971          -        0
      Intr   36216   36177          -        0
      Intr   36347   36300          -        0
      Intr   36722   36567          -        0
      Init   37087   36940          -        0

>3046850        /1680

     len =      580   nex =        2

      Term   44554   44138          -        0
      Init   44695   44629          -        0

>3046850        /27167

     len =     1215   nex =        3

      Term   44554   44109          -        0
      Intr   44695   44629          -        0
      Init   45323   45131          -        0

>3046850        /2557

     len =      790   nex =        3

      Term   58982   58595          -        0
      Intr   59222   59091          -        0
      Init   59377   59312          -        0

>3046850        /21485

     len =     1210   nex =        4

      Init   68376   68477          +        0
      Intr   68594   68717          +        0
      Intr   69144   69216          +        0
      Term   69364   69581          +        0

>3046850        /7347

     len =     1314   nex =        7
```

```
        Term    69704   69481         -         0
        Intr    69872   69789         -         0
        Intr    70063   69955         -         0
        Intr    70270   70147         -         0
        Intr    70434   70338         -         0
        Intr    70647   70515         -         0
        Init    70794   70726         -         0

 >3046850        /13780

      len =     3435    nex =       11

        Term    69704   69439         -         0
        Intr    69872   69789         -         0
        Intr    70063   69955         -         0
        Intr    70270   70147         -         0
        Intr    70434   70338         -         0
        Intr    70647   70515         -         0
        Intr    70803   70726         -         0
        Intr    70979   70872         -         0
        Intr    71133   71060         -         0
        Intr    71700   71616         -         0
        Init    71842   71778         -         0

 >3046851        /39764

      len =     2129    nex =        2

        Init    16125   17251         +         0
        Term    17913   18253         +         0

 >3046851        /19097

      len =     2129    nex =        2

        Init    16148   17251         +         0
        Term    17913   18276         +         0

 >3046851        /39024

      len =     1270    nex =        2

        Init    17002   17251         +         0
        Term    17913   18259         +         0

 >3046851        /108127

      len =     1035    nex =        2

        Init    17225   17251         +         0
        Term    17913   18259         +         0
 >3046851        /42919

      len =     2530    nex =        7

        Init    23586   23813         +         0
        Intr    23901   24521         +         0
        Intr    24672   24859         +         0
        Intr    25008   25246         +         0
```

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```
  Intr   25330   25529        +        0
  Intr   25634   25759        +        0
  Term   25845   26114        +        0

>3046851       /34859

 len =    1887   nex =       6

  Init   24250   24521        +        0
  Intr   24672   24859        +        0
  Intr   25008   25246        +        0
  Intr   25330   25529        +        0
  Intr   25634   25759        +        0
  Term   25845   26136        +        0

>3046851       /103919

 len =     918   nex =       1

  Sngl   47374   48291        +        0

>3046852       /16392

 len =    5153   nex =      13

  Term    5264    5042        -        0
  Intr    5566    5461        -        0
  Intr    5753    5662        -        0
  Intr    6129    6021        -        0
  Intr    6633    6544        -        0
  Intr    7388    7136        -        0
  Intr    7603    7476        -        0
  Intr    7755    7696        -        0
  Intr    8145    8089        -        0
  Intr    8399    8355        -        0
  Intr    8654    8573        -        0
  Intr    8799    8759        -        0
  Init    9050    9006        -        0

>3046852       /30518

 len =    1038   nex =       3

  Init   42931   43061        +        0
  Intr   43292   43357        +        0
  Term   43687   43968        +        0

>3046853       /36632

 len =    1821   nex =       5

  Init   32540   32883        +        0
  Intr   32975   33099        +        0
  Intr   33248   33493        +        0
  Intr   33646   33901        +        0
  Term   33987   34360        +        0

>3046853       /18389

 len =    1810   nex =       5
```

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```
         Init   32589   32883         +         0
         Intr   32975   33099         +         0
         Intr   33248   33493         +         0
         Intr   33646   33901         +         0
         Term   33987   34392         +         0

    >3046853        /21929

       len =      370   nex =        1

         Sngl   34040   34389         +         0

    >3046853        /102017

       len =     1727   nex =        2

         Init   63162   63584         +         0
         Term   64256   64888         +         0

    >3046853        /22470

       len =     1319   nex =        4

         Term   69525   69198         -         0
         Intr   70117   69922         -         0
         Intr   70247   70205         -         0
         Init   70516   70343         -         0

    >3046854        /10299

       len =     1773   nex =        2

         Init   19008   19865         +         0
         Term   20555   20780         +         0

    >3046854        /11612

       len =     1480   nex =        3

         Init   21912   22049         +         0
         Intr   22395   22463         +         0
         Term   22582   23391         +         0

    >3046854        /3994

       len =      730   nex =        2

         Term   23612   23317         -         0
         Init   24037   23700         -         0

    >3046854        /34699

       len =     1554   nex =        3

         Init   38733   39282         +         0
         Intr   39431   39721         +         0
         Term   39804   40286         +         0

    >3046854        /1816
    len =    1677   nex =       6

     Init   57210   57371        +        0
     Intr   57484   57534        +        0
     Intr   57762   57813        +        0
     Intr   58215   58273        +        0
     Intr   58430   58475        +        0
     Term   58598   58886        +        0

>3046854       /37233

    len =    1164   nex =       3

     Term   78862   78599        -        0
     Intr   79238   78963        -        0
     Init   79762   79617        -        0

>3046854       /38961

    len =    1774   nex =       3

     Init    9436    9728        +        0
     Intr    9833   10113        +        0
     Term   10198   10667        +        0

>3046855       /108313

    len =     657   nex =       1

     Sngl   23798   23142        -        0

>3046855       /32984

    len =     970   nex =       1

     Sngl   24087   23142        -        0

>3046855       /14710

    len =    1037   nex =       3

     Init   47198   47333        +        0
     Intr   47668   47769        +        0
     Term   47850   48073        +        0

>3046855       /143364

    len =     697   nex =       3

     Init   47201   47333        +        0
     Intr   47668   47769        +        0
     Term   47850   47897        +        0

>3046855       /115178

    len =    1558   nex =       5

     Init   51391   51541        +        0
     Intr   51756   51855        +        0
```

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```
        Intr   52045   52191          +       0
        Intr   52278   52358          +       0
        Term   52709   52948          +       0

    >3046855        /1440

       len =     743   nex =        3

        Term   61068   60893          -       0
        Intr   61285   61159          -       0
        Init   61635   61504          -       0

    >3046855        /151892

       len =     970   nex =        2

        Term   61285   60730          -       0
        Init   61692   61504          -       0

    >3046855        /20686

       len =     670   nex =        3

        Term   61068   61024          -       0
        Intr   61285   61159          -       0
        Init   61692   61504          -       0

    >3046855        /9633

       len =     937   nex =        3

        Term   61068   60759          -       0
        Intr   61285   61159          -       0
        Init   61695   61504          -       0

    >3046855        /107804

       len =     911   nex =        3

        Term   61068   60785          -       0
        Intr   61285   61159          -       0
        Init   61695   61504          -       0

    >3046855        /21689

       len =    1465   nex =        4

        Init   63956   64174          +       0
        Intr   64250   64483          +       0
        Intr   64572   64658          +       0
        Term   64758   65420          +       0

    >3046855        /92780

       len =     810   nex =        2

        Init   64590   64658          +       0
        Term   64758   65399          +       0

    >3046855        /116709
```

```
   len =      776   nex =        3

    Init   72683   72877          +          0
    Intr   72984   73124          +          0
    Term   73257   73458          +          0

>3046855        /36624

   len =     3759   nex =        4

    Term   74593   73489          -          0
    Intr   75227   74671          -          0
    Intr   76973   76756          -          0
    Init   77247   77060          -          0

>3046856        /19567

   len =     1461   nex =        3

    Init   10176   10234          +          0
    Intr   10433   11125          +          0
    Term   11211   11636          +          0

>3046856        /15527

   len =      550   nex =        1

    Sngl   20236   19688          -          0

>3046856        /103939

   len =     1469   nex =        1

    Sngl   41267   41096          -          0

>3046856        /21741

   len =     1522   nex =        5

    Term   40068   39764          -          0
    Intr   40233   40144          -          0
    Intr   40498   40331          -          0
    Intr   40851   40586          -          0
    Init   41285   41096          -          0

>3046856        /39378

   len =     2380   nex =        7

    Init   42386   42447          +          0
    Intr   42533   42663          +          0
    Intr   42914   42997          +          0
    Intr   43341   43451          +          0
    Intr   43540   43731          +          0
    Intr   43935   44032          +          0
    Term   44339   44765          +          0

>3046856        /25234
```

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```
     len =      839    nex =        2

      Init   43935   44032          +         0
      Term   44339   44773          +         0

>3046856        /7861

     len =     2553    nex =        3

      Term   61214   60794          -         0
      Intr   61683   61434          -         0
      Init   63346   61947          -         0

>3046856        /34558

     len =     2860    nex =        9

      Term   65245   64782          -         0
      Intr   65449   65358          -         0
      Intr   65643   65616          -         0
      Intr   65778   65739          -         0
      Intr   65936   65875          -         0
      Intr   66094   66031          -         0
      Intr   66219   66181          -         0
      Intr   66631   66577          -         0
      Init   66928   66836          -         0

>3046856        /33481

     len =     1393    nex =        3

      Term   74793   73895          -         0
      Intr   75040   74878          -         0
      Init   75287   75126          -         0

>3047060        /107700

     len =     1095    nex =        2

      Init   27099   27748          +         0
      Term   27822   28193          +         0

>3047060        /42538

     len =      675    nex =        1

      Sngl   30075   29401          -         0

>3047074        /30174

     len =      518    nex =        1

      Sngl  102999  102484          -         0

>3047074        /3550

     len =     2660    nex =        6

      Term  104534  103979          -         0
      Intr  104851  104618          -         0
      Intr  105248  104963          -        0
      Intr  105650  105501          -        0
      Intr  105885  105812          -        0
      Init  106638  106431          -        0

>3047074       /21874

    len =     2304    nex =       12

      Term   77864   77757          -        0
      Intr   78105   77960          -        0
      Intr   78294   78189          -        0
      Intr   78454   78377          -        0
      Intr   78603   78550          -        0
      Intr   78757   78697          -        0
      Intr   78979   78855          -        0
      Intr   79155   79069          -        0
      Intr   79339   79266          -        0
      Intr   79549   79420          -        0
      Intr   79716   79634          -        0
      Init   80060   79919          -        0

>3047074       /14379

    len =     1310    nex =        2

      Init   88948   89130          +        0
      Term   89599   90257          +        0

>3047074       /33112

    len =      596    nex =        1

      Sngl   89686   90281          +        0

>3047088       /38281

    len =     1605    nex =        4

      Init   13957   14138          +        0
      Intr   14229   14418          +        0
      Intr   14734   14808          +        0
      Term   14903   15561          +        0

>3047088       /40501

    len =     2311    nex =        4

      Term   16023   15615          -        0
      Intr   16385   16228          -        0
      Intr   16857   16804          -        0
      Init   17417   16963          -        0

>3047088       /20286

    len =     4330    nex =       16

      Init   76297   76485          +        0
      Intr   76590   76926          +        0
      Intr   77004   77145          +        0
```

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```
     Intr   77287   77385        +        0
     Intr   77477   77533        +        0
     Intr   77737   77808        +        0
     Intr   78172   78261        +        0
     Intr   78398   78457        +        0
     Intr   78763   78948        +        0
     Intr   79033   79125        +        0
     Intr   79204   79285        +        0
     Intr   79374   79480        +        0
     Intr   79696   79771        +        0
     Intr   79926   79972        +        0
     Intr   80061   80123        +        0
     Term   80359   80623        +        0

>3047088       /33462

    len =     1594    nex =        7

     Init   79033   79125        +        0
     Intr   79204   79285        +        0
     Intr   79374   79480        +        0
     Intr   79696   79771        +        0
     Intr   79926   79972        +        0
     Intr   80061   80123        +        0
     Term   80359   80623        +        0

>3047100       /34671

    len =     3995    nex =       16

     Init   15513   15554        +        0
     Intr   15672   15764        +        0
     Intr   15953   16020        +        0
     Intr   16133   16208        +        0
     Intr   16307   16353        +        0
     Intr   16466   16582        +        0
     Intr   16799   16876        +        0
     Intr   17024   17111        +        0
     Intr   17262   17374        +        0
     Intr   17513   17575        +        0
     Intr   17785   17889        +        0
     Intr   17986   18045        +        0
     Intr   18316   18417        +        0
     Intr   18618   18666        +        0
     Intr   19056   19124        +        0
     Term   19230   19507        +        0

>3047100       /9233

    len =     1124    nex =        2

     Init   19593   19904        +        0
     Term   20386   20716        +        0

>3047100       /31467

    len =      730    nex =        2

     Term   20944   20738        -        0
     Init   21458   21292        -        0
```

09555368.061502

```
>3047100        /18459

    len =     1570    nex =        1

      Sngl    39059    38782          -         0

>3047100        /18629

    len =     1636    nex =        1

      Sngl    39059    38776          -         0

>3047100        /545

    len =      498    nex =        2

      Term    67746    67593          -         0
      Init    68090    67824          -         0

>3047100        /26070

    len =     1097    nex =        3

      Term    67746    67416          -         0
      Intr    68089    67824          -         0
      Init    68512    68419          -         0

>3047100        /28642

    len =     3207    nex =        9

      Init    78779    78999          +         0
      Intr    79083    79218          +         0
      Intr    79317    79450          +         0
      Intr    79542    79661          +         0
      Intr    79739    79824          +         0
      Intr    79921    80109          +         0
      Intr    80200    80352          +         0
      Intr    80464    80577          +         0
      Term    80658    81268          +         0

>3056579        /158942

    len =      831    nex =        1

      Sngl    32993    33823          +         0

>3056579        /13461

    len =     3450    nex =        7

      Init    42933    44316          +         0
      Intr    44426    44536          +         0
      Intr    44764    44900          +         0
      Intr    44977    45187          +         0
      Intr    45292    45529          +         0
      Intr    45624    45774          +         0
      Term    45872    46382          +         0
```

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```
>3056579        /38645

   len =    1408   nex =      3

    Term   62928   62777        -        0
    Intr   63094   63020        -        0
    Init   63938   63662        -        0

>3059018        /29133

   len =    1298   nex =      2

    Term   19437   19300        -        0
    Init   19744   19558        -        0

>3059018        /20592

   len =    2679   nex =      4

    Init   70884   70974        +        0
    Intr   72180   72269        +        0
    Intr   72361   72445        +        0
    Term   72984   73562        +        0

>3059018        /38430

   len =    1379   nex =      1

    Sngl   72180   72270        +        0

>3059018        /38689

   len =     610   nex =      1

    Sngl   82744   83198        +        0

>3059018        /18947

   len =    1732   nex =      3

    Init   87586   88234        +        0
    Intr   88340   88417        +        0
    Term   88957   89317        +        0

>3063438        /7188

   len =     861   nex =      1

    Sngl  106806  107666        +        0

>3063438        /32337

   len =    1841   nex =      4

    Init   10816   11218        +        0
    Intr   11470   11646        +        0
    Intr   11732   11896        +        0
    Term   11987   12656        +        0

>3063438        /20276
```

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```
      len =     1412    nex =        3

        Term  108108   107703          -         0
        Intr  108949   108833          -         0
        Init  109114   109038          -         0

  >3063438        /33493

      len =     2171    nex =        7

        Init  125603   125698          +         0
        Intr  125795   125896          +         0
        Intr  126029   126124          +         0
        Intr  126303   126334          +         0
        Intr  126429   126504          +         0
        Intr  126676   126729          +         0
        Term  126954   127179          +         0

  >3063438        /118748

      len =     2133    nex =        7

        Init  125603   125698          +         0
        Intr  125795   125896          +         0
        Intr  126029   126124          +         0
        Intr  126303   126334          +         0
        Intr  126429   126504          +         0
        Intr  126676   126729          +         0
        Term  126954   127173          +         0

  >3063438        /36523

      len =     2136    nex =        7

        Init  125603   125698          +         0
        Intr  125795   125896          +         0
        Intr  126029   126124          +         0
        Intr  126303   126334          +         0
        Intr  126429   126504          +         0
        Intr  126676   126729          +         0
        Term  126954   127176          +         0

  >3063438        /34741

      len =     1210    nex =        2

        Term   13932    13866          -         0
        Init   14277    14113          -         0

  >3063438        /35718

      len =     2841    nex =        9

        Term   47185    47114          -         0
        Intr   47500    47405          -         0
        Intr   47724    47599          -         0
        Intr   47999    47942          -         0
        Intr   48148    48093          -         0
        Intr   48315    48238          -         0
```

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```
      Intr   48516    48412        -        0
      Intr   48701    48602        -        0
      Init   49201    49002        -        0

>3063438        /43026

     len =    1060    nex =       1

      Sngl   68860    67801        -        0

>3063438        /27805

     len =    1272    nex =       1

      Sngl    8642     7563        -        0

>3063690        /40949

     len =    2193    nex =       3

      Init   18843    19345        +        0
      Intr   19886    19966        +        0
      Term   20222    21035        +        0

>3063690        /18482

     len =    3096    nex =       5

      Term   21537    21211        -        0
      Intr   21947    21729        -        0
      Intr   22174    22060        -        0
      Intr   22529    22282        -        0
      Init   24306    22854        -        0

>3063690        /35221

     len =    1829    nex =       2

      Init   51969    52232        +        0
      Term   52962    53797        +        0

>3063690        /39535

     len =    1673    nex =       3

      Init   89115    89369        +        0
      Intr   89531    89856        +        0
      Term   90448    90787        +        0

>3063690        /28609

     len =    1510    nex =       3

      Term   94514    94452        -        0
      Intr   95366    95253        -        0
      Init   95700    95452        -        0

>3068702        /21630

     len =    1352    nex =       4
```

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```
     Term    9605    9217        -       0
     Intr    9840    9694        -       0
     Intr   10174    9918        -       0
     Init   10568   10350        -       0

>3068702        /38567

    len =     1554   nex =        4

     Term    9605    9176        -       0
     Intr    9840    9694        -       0
     Intr   10174    9918        -       0
     Init   10729   10350        -       0

>3068702        /6926

    len =      872   nex =        2

     Term   12518   12287        -       0
     Init   13158   12549        -       0

>3068702        /3844

    len =     3670   nex =       12

     Term   28154   27776        -       0
     Intr   28332   28240        -       0
     Intr   28985   28814        -       0
     Intr   29153   29077        -       0
     Intr   29837   29728        -       0
     Intr   30000   29941        -       0
     Intr   30183   30110        -       0
     Intr   30416   30293        -       0
     Intr   30579   30502        -       0
     Intr   30782   30676        -       0
     Intr   30970   30866        -       0
     Init   31445   31360        -       0

>3068702        /93427

    len =     1415   nex =        3

     Term   52373   51897        -       0
     Intr   52618   52465        -       0
     Init   53311   52956        -       0

>3075383        /37427

    len =     2556   nex =        3

     Init   47142   47616        +       0
     Intr   48151   48776        +       0
     Term   48851   49697        +       0

>3075383        /4752

    len =      562   nex =        1

     Sngl   49196   49757        +       0
```

```
>3075383        /33535

   len =     1150    nex =        3

     Init   51795    51949          +         0
     Intr   52409    52543          +         0
     Term   52636    52942          +         0

>3075383        /38538

   len =     2558    nex =        8

     Term   53383    53089          -         0
     Intr   53656    53563          -         0
     Intr   53916    53743          -         0
     Intr   54085    53994          -         0
     Intr   54632    54523          -         0
     Intr   54787    54712          -         0
     Intr   54992    54900          -         0
     Init   55322    55106          -         0

>3080352        /18098

   len =     1590    nex =        8

     Term   40284    40098          -         0
     Intr   40461    40369          -         0
     Intr   40639    40558          -         0
     Intr   40838    40768          -         0
     Intr   40960    40928          -         0
     Intr   41121    41092          -         0
     Intr   41295    41227          -         0
     Init   41687    41583          -         0

>3080352        /38902

   len =     1694    nex =        8

     Term   40284    40037          -         0
     Intr   40461    40369          -         0
     Intr   40639    40558          -         0
     Intr   40838    40768          -         0
     Intr   40960    40928          -         0
     Intr   41121    41092          -         0
     Intr   41295    41227          -         0
     Init   41730    41583          -         0

>3080352        /39492

   len =     1192    nex =        1

     Sngl   56521    57712          +         0

>3080352        /119432

   len =     1076    nex =        4

     Term   59135    58928          -         0
     Intr   59332    59218          -         0
```

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```
    Intr   59524   59415        -       0
    Init   60003   59857        -       0

>3080352       /34674

  len =    3652   nex =      12

    Term   60315   60098        -       0
    Intr   60539   60394        -       0
    Intr   60747   60642        -       0
    Intr   60927   60850        -       0
    Intr   61080   61027        -       0
    Intr   61235   61175        -       0
    Intr   61529   61405        -       0
    Intr   61701   61615        -       0
    Intr   61918   61839        -       0
    Intr   62170   62041        -       0
    Intr   62546   62464        -       0
    Init   62781   62669        -       0

>3080352       /11264

  len =    2126   nex =      10

    Term   65646   65231        -       0
    Intr   65897   65752        -       0
    Intr   66105   66000        -       0
    Intr   66302   66225        -       0
    Intr   66445   66392        -       0
    Intr   66599   66539        -       0
    Intr   66819   66695        -       0
    Intr   67006   66920        -       0
    Intr   67165   67086        -       0
    Init   67356   67258        -       0

>3080352       /36325

  len =    3216   nex =      12

    Term   65646   65229        -       0
    Intr   65897   65752        -       0
    Intr   66105   66000        -       0
    Intr   66302   66225        -       0
    Intr   66445   66392        -       0
    Intr   66599   66539        -       0
    Intr   66819   66695        -       0
    Intr   67006   66920        -       0
    Intr   67165   67086        -       0
    Intr   67387   67258        -       0
    Intr   67607   67525        -       0
    Init   67822   67707        -       0

>3080352       /13917

  len =     681   nex =       1

    Sngl   77159   76479        -       0

>3080406       /32793
```

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```
    len =     1690    nex =        5

     Init     3323     3465         +          0
     Intr     3592     3780         +          0
     Intr     3945     4107         +          0
     Intr     4211     4403         +          0
     Term     4497     4800         +          0

>3080406         /37017

    len =     2965    nex =       11

     Term    28768    28521         -          0
     Intr    29006    28912         -          0
     Intr    29136    29088         -          0
     Intr    29290    29231         -          0
     Intr    29583    29462         -          0
     Intr    29930    29882         -          0
     Intr    30171    30100         -          0
     Intr    30408    30308         -          0
     Intr    31062    30951         -          0
     Intr    31203    31134         -          0
     Init    31485    31423         -          0

>3080406         /22906

    len =     1493    nex =        5

     Term     5013     4742         -          0
     Intr     5195     5109         -          0
     Intr     5342     5307         -          0
     Intr     5651     5451         -          0
     Init     5811     5725         -          0

>3080406         /12228

    len =     2116    nex =        4

     Init    62410    63011         +          0
     Intr    63132    63371         +          0
     Intr    63788    63866         +          0
     Term    63967    64525         +          0

>3080406         /2912

    len =     2158    nex =        7

     Init    65401    65852         +          0
     Intr    65941    66039         +          0
     Intr    66212    66406         +          0
     Intr    66528    66620         +          0
     Intr    66715    66810         +          0
     Intr    66903    67043         +          0
     Term    67158    67558         +          0

>3080406         /1368

    len =     1665    nex =        6

     Term    84894    84458         -          0
```

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```
    Intr   85064   84994        -       0
    Intr   85285   85188        -       0
    Intr   85426   85354        -       0
    Intr   85611   85567        -       0
    Init   86122   85694        -       0

>3080430       /14423

   len =     464   nex =        1

    Sngl   16915   17378        +       0

>3080430       /21166

   len =    1789   nex =        6

    Term     903     736        -       0
    Intr    1133     998        -       0
    Intr    1450    1388        -       0
    Intr    1804    1758        -       0
    Intr    2094    1882        -       0
    Init    2524    2295        -       0

>3080430       /5968

   len =     759   nex =        2

    Init   36053   36158        +       0
    Term   36245   36811        +       0

>3080430       /38152

   len =    1829   nex =        5

    Term   38834   38436        -       0
    Intr   39547   39329        -       0
    Intr   39701   39626        -       0
    Intr   39938   39797        -       0
    Init   40254   40174        -       0

>3080430       /1978

   len =    2536   nex =        9

    Term   72981   72569        -       0
    Intr   73256   73068        -       0
    Intr   73521   73345        -       0
    Intr   73732   73617        -       0
    Intr   74041   73815        -       0
    Intr   74377   74130        -       0
    Intr   74609   74474        -       0
    Intr   74761   74693        -       0
    Init   75104   74843        -       0

>3080430       /6464

   len =    1702   nex =        2

    Term   79588   79377        -       0
    Init   81078   80640        -       0
```

```
>3080430        /17227

   len =     134    nex =       1

    Sngl    9916    10049         +        0

>3108024        /3434

   len =    2352    nex =       2

    Term   29167    28743         -        0
    Init   30389    29273         -        0

>3108024        /124122

   len =    1576    nex =       7

    Term   43805    43635         -        0
    Intr   43963    43896         -        0
    Intr   44150    44048         -        0
    Intr   44473    44243         -        0
    Intr   44946    44906         -        0
    Intr   45067    45031         -        0
    Init   45200    45177         -        0

>3108024        /2164

   len =    1615    nex =       7

    Term   43805    43610         -        0
    Intr   43963    43896         -        0
    Intr   44150    44048         -        0
    Intr   44473    44243         -        0
    Intr   44946    44906         -        0
    Intr   45067    45031         -        0
    Init   45224    45177         -        0

>3108025        /34936

   len =    2501    nex =       4

    Init  101875   102049         +        0
    Intr  102345   102824         +        0
    Intr  102969   103448         +        0
    Term  103539   104375         +        0

>3108025        /113281

   len =    1612    nex =       3

    Term  104623   104358         -        0
    Intr  105265   104996         -        0
    Init  105449   105341         -        0

>3128134        /33790

   len =    2267    nex =       4

    Init   16068    16558         +        0
    Intr   17365   17675        +        0
    Intr   17756   17820        +        0
    Term   17936   18334        +        0

>3128134        /37644

   len =     2174    nex =        6

    Term   19620   18952        -        0
    Intr   19787   19706        -        0
    Intr   20141   20058        -        0
    Intr   20421   20245        -        0
    Intr   20669   20507        -        0
    Init   21125   20761        -        0

>3128134        /42970

   len =     1721    nex =        2

    Init   22577   22849        +        0
    Term   22987   24297        +        0

>3128134        /39030

   len =      619    nex =        1

    Sngl    6536    7154        +        0

>3128135        /33791

   len =     1822    nex =        2

    Init   21696   22080        +        0
    Term   22757   23517        +        0

>3128135        /25162

   len =     1581    nex =        4

    Term   47134   46795        -        0
    Intr   47507   47403        -        0
    Intr   47751   47624        -        0
    Init   48188   48109        -        0

>3128135        /39130

   len =     1611    nex =        5

    Term   47134   46806        -        0
    Intr   47507   47403        -        0
    Intr   47751   47624        -        0
    Intr   48188   48109        -        0
    Init   48416   48288        -        0

>3128135        /18718

   len =     1690    nex =        3

    Init   48812   49068        +        0
    Intr   49281   49599        +        0
   Term   50104   50162         +        0

>3128135       /110653

  len =    1658   nex =       5

   Init   53406   53626         +        0
   Intr   53710   53762         +        0
   Intr   53845   54012         +        0
   Intr   54431   54538         +        0
   Term   54771   55063         +        0

>3128135       /1455

  len =    2771   nex =      11

   Term   55477   55365         -        0
   Intr   55672   55601         -        0
   Intr   55818   55768         -        0
   Intr   56007   55920         -        0
   Intr   56234   56111         -        0
   Intr   56410   56338         -        0
   Intr   56574   56511         -        0
   Intr   56855   56662         -        0
   Intr   56992   56943         -        0
   Intr   57191   57128         -        0
   Init   57772   57284         -        0

>3128135       /6216

  len =    3834   nex =      15

   Init   58058   58788         +        0
   Intr   58941   59002         +        0
   Intr   59123   59223         +        0
   Intr   59534   59584         +        0
   Intr   59679   59753         +        0
   Intr   59861   59938         +        0
   Intr   60097   60153         +        0
   Intr   60287   60338         +        0
   Intr   60449   60519         +        0
   Intr   60620   60719         +        0
   Intr   60816   60920         +        0
   Intr   61011   61073         +        0
   Intr   61154   61276         +        0
   Intr   61370   61531         +        0
   Term   61621   61891         +        0

>3128135       /235

  len =    1547   nex =       3

   Init   62091   62463         +        0
   Intr   62667   63009         +        0
   Term   63093   63637         +        0

>3128136       /35906

  len =     738   nex =       1
```

```
    Sngl        1      738          +        0

>3128136        /3080

   len =     1342    nex =        4

    Init   23530    24049          +        0
    Intr   24161    24386          +        0
    Intr   24474    24547          +        0
    Term   24640    24871          +        0

>3128136        /2222

   len =     1300    nex =        4

    Init   23572    24049          +        0
    Intr   24161    24386          +        0
    Intr   24474    24547          +        0
    Term   24640    24871          +        0

>3128136        /38030

   len =     2477    nex =        5

    Term   28271    27313          -        0
    Intr   28564    28362          -        0
    Intr   28798    28676          -        0
    Intr   29081    28887          -        0
    Init   29789    29424          -        0

>3128136        /93374

   len =      550    nex =        1

    Sngl   36684    36143          -        0

>3128136        /34700

   len =     1901    nex =        5

    Term   39171    38646          -        0
    Intr   39414    39263          -        0
    Intr   39628    39503          -        0
    Intr   39879    39736          -        0
    Init   40546    40298          -        0

>3128136        /35310

   len =     1990    nex =        9

    Term   42802    42663          -        0
    Intr   42973    42892          -        0
    Intr   43171    43064          -        0
    Intr   43366    43269          -        0
    Intr   43569    43471          -        0
    Intr   43782    43645          -        0
    Intr   43925    43874          -        0
    Intr   44136    44023          -        0
    Init   44318    44220          -        0
```

```
>3128136         /15350

   len =     3109    nex =       11

    Term   45887    45746          -          0
    Intr   46034    45984          -          0
    Intr   46201    46130          -          0
    Intr   46384    46316          -          0
    Intr   46520    46479          -          0
    Intr   46680    46612          -          0
    Intr   46901    46764          -          0
    Intr   47104    46994          -          0
    Intr   47275    47188          -          0
    Intr   47451    47396          -          0
    Init   48367    47549          -          0

>3128136         /37218

   len =     1584    nex =        3

    Init   57574    57902          +          0
    Intr   58199    58492          +          0
    Term   58579    59157          +          0

>3128136         /149202

   len =     1438    nex =        2

    Init   57626    57902          +          0
    Term   58579    59063          +          0

>3128136         /31524

   len =      750    nex =        1

    Sngl   61551    62300          +          0

>3128136         /2152

   len =      681    nex =        1

    Sngl   63097    63777          +          0

>3128136         /2489

   len =     1832    nex =        7

    Init    8722     8908          +          0
    Intr    9213     9287          +          0
    Intr    9396     9443          +          0
    Intr    9532     9688          +          0
    Intr    9777     9849          +          0
    Intr   10088    10185          +          0
    Term   10266    10553          +          0

>3128137         /1311

   len =      670    nex =        1

    Sngl   10381    10111          -          0
```

```
>3128137        /2518

  len =      509    nex =        1

   Sngl   13914   13406         -         0

>3128137        /2561

  len =      639    nex =        1

   Sngl   17617   16979         -         0

>3128137        /35979

  len =     1795    nex =        6

   Init   29554   29782         +         0
   Intr   30223   30304         +         0
   Intr   30392   30424         +         0
   Intr   30505   30626         +         0
   Intr   30713   30874         +         0
   Term   30962   31348         +         0

>3128137        /25262

  len =     2145    nex =        4

   Term   35368   34765         -         0
   Intr   35549   35466         -         0
   Intr   35707   35623         -         0
   Init   36012   35792         -         0

>3128137        /28455

  len =     1527    nex =        5

   Init   37172   37397         +         0
   Intr   37545   37633         +         0
   Intr   37818   37947         +         0
   Intr   38129   38158         +         0
   Term   38466   38698         +         0

>3128137        /9946

  len =      719    nex =        2

   Term   39259   39103         -         0
   Init   39475   39347         -         0

>3128138        /14654

  len =      953    nex =        4

   Term   33774   33527         -         0
   Intr   33934   33895         -         0
   Intr   34248   34172         -         0
   Init   34479   34388         -         0

>3128139        /6095
```

```
     len =      203    nex =        1

      Sngl   34265   34183         -         0

  >3128139         /9938

     len =      769    nex =        3

      Term   33664   33501         -         0
      Intr   34085   33948         -         0
      Init   34269   34183         -         0

  >3128139         /114411

     len =      835    nex =        4

      Init   36332   36381         +         0
      Intr   36467   36596         +         0
      Intr   36706   36764         +         0
      Term   36863   37166         +         0

  >3128139         /100570

     len =      408    nex =        1

      Sngl   42429   42022         -         0

  >3128139         /30782

     len =     1108    nex =        0

  >3128139         /14992

     len =     1135    nex =        1

      Sngl   42487   42041         -         0

  >3128139         /117908

     len =      101    nex =        1

      Sngl   53867   53767         -         0

  >3128139         /36495

     len =     3037    nex =       10

      Init   56082   56250         +         0
      Intr   57096   57195         +         0
      Intr   57270   57367         +         0
      Intr   57458   57546         +         0
      Intr   57631   57691         +         0
      Intr   57783   57947         +         0
      Intr   58040   58096         +         0
      Intr   58194   58249         +         0
      Intr   58361   58496         +         0
      Term   58593   59118         +         0

  >3128139         /119783
```

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```
     len =     1306    nex =        2

      Term    60197    59560       -         0
      Init    60865    60344       -         0

  >3128139        /2640

     len =     1630    nex =        3

      Init    78927    79261       +         0
      Intr    79585    79788       +         0
      Term    80124    80547       +         0

  >3128139        /102364

     len =     1119    nex =        3

      Term     8221     7893       -         0
      Intr     8425     8288       -         0
      Init     9011     8793       -         0

  >3128140        /23770

     len =     1631    nex =        5

      Term    36206    35989       -         0
      Intr    36361    36296       -         0
      Intr    36935    36804       -         0
      Intr    37208    37034       -         0
      Init    37619    37369       -         0

  >3128140        /4372

     len =     2792    nex =       10

      Init    42434    42735       +         0
      Intr    42817    42920       +         0
      Intr    42997    43045       +         0
      Intr    43129    43235       +         0
      Intr    43321    43408       +         0
      Intr    43491    43619       +         0
      Intr    43701    43775       +         0
      Intr    43849    44100       +         0
      Intr    44187    44247       +         0
      Term    44328    44635       +         0

  >3128141        /11386

     len =      647    nex =        1

      Sngl    27315    26669       -         0

  >3128141        /218

     len =      610    nex =        1

      Sngl    28622    28020       -         0

  >3128141        /41397
```

```
      len =     1330    nex =        1

       Sngl   38776   37454        -         0

>3128141         /155962

      len =      618    nex =        1

       Sngl    5415    4798        -         0

>3128141         /31445

      len =     1875    nex =        2

       Init   58824   59151        +         0
       Term   60191   60698        +         0

>3128142         /29670

      len =     1810    nex =        8

       Term   18523   18377        -         0
       Intr   18697   18620        -         0
       Intr   18917   18816        -         0
       Intr   19057   19001        -         0
       Intr   19247   19152        -         0
       Intr   19419   19348        -         0
       Intr   19665   19525        -         0
       Init   20185   19759        -         0

>3128142         /20783

      len =     2186    nex =        8

       Init   43584   43663        +         0
       Intr   43850   44097        +         0
       Intr   44207   44257        +         0
       Intr   44381   44536        +         0
       Intr   44649   44805        +         0
       Intr   44887   45029        +         0
       Intr   45105   45140        +         0
       Term   45266   45769        +         0

>3128142         /116956

      len =     1195    nex =        2

       Term   49243   48803        -         0
       Init   49997   49329        -         0

>3128142         /10051

      len =     1117    nex =        3

       Term   49243   48881        -         0
       Intr   49488   49329        -         0
       Init   49997   49638        -         0

>3128142         /40335
```

```
  len =      398    nex =        1

   Sngl   50607   50210         -        0

>3128142        /31032

  len =     3178    nex =        9

   Init   55780   56016         +        0
   Intr   56983   57164         +        0
   Intr   57277   57357         +        0
   Intr   57442   57501         +        0
   Intr   57586   57663         +        0
   Intr   57752   57815         +        0
   Intr   57898   58169         +        0
   Intr   58258   58485         +        0
   Term   58560   58957         +        0

>3128142        /16740

  len =     1431    nex =        2

   Init   59192   59602         +        0
   Term   60287   60622         +        0

>3128142        /30437

  len =     1315    nex =        2

   Init   81339   81632         +        0
   Term   82066   82653         +        0

>3128142        /8294

  len =     1150    nex =        2

   Term   84674   84011         -        0
   Init   85146   84757         -        0

>3128143        /39401

  len =     3813    nex =       11

   Init   16973   17402         +        0
   Intr   17496   17719         +        0
   Intr   17842   18089         +        0
   Intr   18179   18398         +        0
   Intr   18712   18839         +        0
   Intr   19099   19355         +        0
   Intr   19458   19565         +        0
   Intr   19668   19874         +        0
   Intr   19978   20088         +        0
   Intr   20176   20349         +        0
   Term   20425   20785         +        0

>3128143        /24169

  len =     1371    nex =        2
```

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```
    Term    21558    21040          -          0
    Init    22410    21729          -          0

>3128143        /22388

    len =    1134    nex =        3

    Init    23440    23527          +          0
    Intr    23989    24077          +          0
    Term    24347    24573          +          0

>3128143        /97304

    len =    1618    nex =        4

    Init    34719    34756          +          0
    Intr    35037    35222          +          0
    Intr    35369    35408          +          0
    Term    35792    36036          +          0

>3128143        /17995

    len =    2075    nex =        3

    Init    38456    38783          +          0
    Intr    38871    39113          +          0
    Term    39198    40530          +          0

>3128143        /5688

    len =     518    nex =        1

    Sngl    43484    44001          +          0

>3128143        /41898

    len =     416    nex =        1

    Sngl    43585    44000          +          0

>3128143        /11837

    len =    2078    nex =        8

    Term    44515    44311          -          0
    Intr    44724    44603          -          0
    Intr    44933    44823          -          0
    Intr    45130    45020          -          0
    Intr    45250    45213          -          0
    Intr    45393    45323          -          0
    Intr    46203    46049          -          0
    Init    46388    46280          -          0

>3128143        /6495

    len =    2273    nex =        8

    Term    44515    44170          -          0
    Intr    44724    44603          -          0
    Intr    44933    44823          -          0
```

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```
    Intr   45130   45020        -        0
    Intr   45250   45213        -        0
    Intr   45393   45323        -        0
    Intr   46203   46049        -        0
    Init   46442   46280        -        0

>3128143       /43069

  len =     1223   nex =       2

    Term   47252   46474        -        0
    Init   47696   47350        -        0

>3128143       /31005

  len =     1417   nex =       4

    Term   48228   47939        -        0
    Intr   48546   48312        -        0
    Intr   48718   48648        -        0
    Init   49355   49116        -        0

>3128143       /4502

  len =     1283   nex =       1

    Sngl   54087   54642        +        0

>3128143       /3836

  len =     1672   nex =       5

    Init   57472   57668        +        0
    Intr   57754   57814        +        0
    Intr   58106   58210        +        0
    Intr   58389   58672        +        0
    Term   58870   59134        +        0

>3128143       /5915

  len =      563   nex =       3

    Init   78978   79080        +        0
    Intr   79169   79306        +        0
    Term   79392   79540        +        0

>3128166       /34876

  len =     2304   nex =       7

    Init  104985  105384        +        0
    Intr  105472  105604        +        0
    Intr  105754  105891        +        0
    Intr  105992  106125        +        0
    Intr  106247  106372        +        0
    Intr  106603  106706        +        0
    Term  107114  107288        +        0

>3128166       /8402
```

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```
  len =    1414   nex =       5

   Init  107501  107707        +        0
   Intr  107864  107980        +        0
   Intr  108265  108299        +        0
   Intr  108441  108541        +        0
   Term  108639  108914        +        0

>3128166       /9014

  len =    2503   nex =       8

   Init  109037  109232        +        0
   Intr  109367  109543        +        0
   Intr  109771  109860        +        0
   Intr  110290  110433        +        0
   Intr  110541  110708        +        0
   Intr  110780  110849        +        0
   Intr  110930  111011        +        0
   Term  111101  111260        +        0

>3128166       /91801

  len =     655   nex =       2

   Term   23650   23349        -        0
   Init   23856   23745        -        0

>3128166       /18124

  len =    2278   nex =      10

   Term   23650   23314        -        0
   Intr   23856   23745        -        0
   Intr   24003   23937        -        0
   Intr   24158   24112        -        0
   Intr   24328   24263        -        0
   Intr   24527   24415        -        0
   Intr   24742   24623        -        0
   Intr   24972   24830        -        0
   Intr   25156   25042        -        0
   Init   25591   25463        -        0

>3128166       /2895

  len =     705   nex =       2

   Term   31097   30635        -        0
   Init   31334   31265        -        0

>3128166       /26701

  len =     599   nex =       1

   Sngl   34617   35215        +        0

>3128166       /16528

  len =    4456   nex =      19
```

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```
        Init    42061    42128          +         0
        Intr    42230    42292          +         0
        Intr    42439    42480          +         0
        Intr    42662    42727          +         0
        Intr    42820    42885          +         0
        Intr    43008    43121          +         0
        Intr    43230    43293          +         0
        Intr    43471    43553          +         0
        Intr    43682    43731          +         0
        Intr    43811    43888          +         0
        Intr    44033    44161          +         0
        Intr    44296    44338          +         0
        Intr    44430    44516          +         0
        Intr    44684    44785          +         0
        Intr    45134    45241          +         0
        Intr    45333    45383          +         0
        Intr    45531    45604          +         0
        Intr    45682    45733          +         0
        Term    45831    46160          +         0

   >3128166        /40257

        len =      474    nex =        1

        Sngl    47811    47358          -         0

   >3128166        /23768

        len =      970    nex =        0

   >3128166        /21043

        len =     2437    nex =        9

        Init    55878    56041          +         0
        Intr    56251    56433          +         0
        Intr    56538    56602          +         0
        Intr    56676    56775          +         0
        Intr    56882    56920          +         0
        Intr    57009    57191          +         0
        Intr    57460    57638          +         0
        Intr    57783    57902          +         0
        Term    57996    58314          +         0

   >3128166        /39057

        len =     1992    nex =        7

        Init     6113     6363          +         0
        Intr     6454     6540          +         0
        Intr     6632     6895          +         0
        Intr     6985     7149          +         0
        Intr     7228     7281          +         0
        Intr     7374     7520          +         0
        Term     7616     8104          +         0

   >3132469        /14874

        len =     2445    nex =        6
```

```
      Term    4589     4297        -         0
      Intr    4838     4692        -         0
      Intr    5021     4916        -         0
      Intr    5351     5155        -         0
      Intr    5552     5460        -         0
      Init    6741     5705        -         0

 >3133272        /15372

     len =      596    nex =        1

      Sngl   15821    16416         +         0

 >3133272        /105334

     len =      190    nex =        1

      Sngl   47085    47265         +         0

 >3135250        /99763

     len =      598    nex =        1

      Sngl   34775    34178         -         0

 >3135250        /37787

     len =     1140    nex =        2

       Init   40584    40900         +         0
       Term   41358    41723         +         0

 >3150395        /3754

     len =      708    nex =        1

      Sngl   12833    13540         +         0

 >3150395        /37527

     len =     1938    nex =        5

      Term   19470    19016         -         0
      Intr   19706    19545         -         0
      Intr   20319    19790         -         0
      Intr   20522    20409         -         0
      Init   20953    20632         -         0

 >3150395        /34995

     len =     2070    nex =        5

      Term   21605    21212         -         0
      Intr   21821    21733         -         0
      Intr   22028    21935         -         0
      Intr   22801    22629         -         0
      Init   23281    23141         -         0

 >3150395        /106011
```

```
    len =     2110   nex =        5

     Term   21605   21183        -         0
     Intr   21821   21733        -         0
     Intr   22028   21935        -         0
     Intr   22801   22629        -         0
     Init   23286   23141        -         0

>3150395        /22034

    len =     1351   nex =        3

     Term   23931   23690        -         0
     Intr   24424   24224        -         0
     Init   25040   24615        -         0

>3150395        /1853

    len =     1297   nex =        3

     Init   75585   75743        +         0
     Intr   75875   75961        +         0
     Term   76230   76442        +         0

>3150396        /299

    len =     2574   nex =       10

     Init   20107   20448        +         0
     Intr   20697   20782        +         0
     Intr   20860   20926        +         0
     Intr   21087   21229        +         0
     Intr   21397   21445        +         0
     Intr   21591   21728        +         0
     Intr   21813   21893        +         0
     Intr   21979   22155        +         0
     Intr   22242   22328        +         0
     Term   22416   22680        +         0

>3150396        /37777

    len =     1499   nex =        7

     Init   21157   21229        +         0
     Intr   21397   21445        +         0
     Intr   21591   21728        +         0
     Intr   21813   21893        +         0
     Intr   21979   22155        +         0
     Intr   22242   22328        +         0
     Term   22416   22655        +         0

>3150396        /18023

    len =      550   nex =        1

     Sngl   29680   30224        +         0

>3150396        /29970

    len =     1095   nex =        2
```

```
        Term   36375   35916         -        0
        Init   37010   36739         -        0

   >3150396       /4576

      len =     1543   nex =       2

        Init   65489   66226         +        0
        Term   66584   67031         +        0

   >3150396       /29581

      len =     1572   nex =       4

        Term   77303   77124         -        0
        Intr   78071   77946         -        0
        Intr   78321   78170         -        0
        Init   78695   78445         -        0

   >3150396       /36577

      len =     2177   nex =       1

        Sngl   80119   79013         -        0

   >3152602       /33637

      len =     1336   nex =       2

        Init   12908   13530         +        0
        Term   13557   14243         +        0

   >3152602       /17402

      len =     1296   nex =       1

        Sngl   16191   17124         +        0

   >3152602       /2328

      len =      651   nex =       1

        Sngl   68201   67551         -        0

   >3152602       /36311

      len =     1291   nex =       1

        Sngl    7655    8945         +        0

   >3152602       /20125

      len =      690   nex =       1

        Sngl   76571   77260         +        0

   >3152602       /104289

      len =      760   nex =       1
```

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```
    Sngl   77189   76430         -         0

>3169169        /41424

   len =     4293    nex =       10

    Init   10438   10717         +         0
    Intr   10800   10945         +         0
    Intr   11012   11088         +         0
    Intr   11168   11290         +         0
    Intr   11602   12013         +         0
    Intr   12378   12464         +         0
    Intr   12552   12620         +         0
    Intr   12727   12798         +         0
    Intr   14190   14303         +         0
    Term   14407   14730         +         0

>3169169        /12708

   len =     4175    nex =       12

    Init   10565   10717         +         0
    Intr   10800   10945         +         0
    Intr   11012   11088         +         0
    Intr   11168   11290         +         0
    Intr   11602   11704         +         0
    Intr   11912   12013         +         0
    Intr   12378   12464         +         0
    Intr   12552   12620         +         0
    Intr   12727   12798         +         0
    Intr   13609   13730         +         0
    Intr   14190   14303         +         0
    Term   14559   14739         +         0

>3169169        /33165

   len =     3649    nex =       14

    Init   23773   23969         +         0
    Intr   24056   24201         +         0
    Intr   24279   24355         +         0
    Intr   24441   24560         +         0
    Intr   24831   24933         +         0
    Intr   25023   25060         +         0
    Intr   25156   25257         +         0
    Intr   25439   25525         +         0
    Intr   25617   25700         +         0
    Intr   25899   25970         +         0
    Intr   26085   26203         +         0
    Intr   26875   26988         +         0
    Intr   27084   27133         +         0
    Term   27222   27421         +         0

>3169169        /38084

   len =     2296    nex =       10

    Init   58707   58874         +         0
    Intr   59120   59249         +         0
```

```
           Intr    59331    59525          +        0
           Intr    59614    59771          +        0
           Intr    59925    60015          +        0
           Intr    60148    60213          +        0
           Intr    60303    60385          +        0
           Intr    60504    60567          +        0
           Intr    60639    60707          +        0
           Term    60845    61002          +        0

     >3169169         /24000

          len =      850    nex =        3

           Term    61337    61068          -        0
           Intr    61652    61612          -        0
           Init    61908    61760          -        0

     >3169169         /14468

          len =      953    nex =        3

           Term    61337    61019          -        0
           Intr    61652    61612          -        0
           Init    61971    61760          -        0

     >3169169         /27437

          len =      407    nex =        1

            Sngl   73602    74008          +        0

     >3169169         /206407

          len =      478    nex =        1

            Sngl   73603    74080          +        0

     >3169169         /9937

          len =      478    nex =        1

            Sngl   73604    74081          +        0

     >3169169         /28423

          len =      507    nex =        1

            Sngl   73604    74110          +        0

     >3169169         /26935

          len =      530    nex =        1

            Sngl   73604    74133          +        0

     >3169169         /11891

          len =      532    nex =        1

            Sngl   73604    74135          +        0
```

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```
>3169169        /121073

   len =      478    nex =        1

    Sngl   73656   74133         +         0

>3172156        /26867

   len =      790    nex =        1

    Sngl    3280    4062         +         0

>3172156        /7894

   len =     2323    nex =        4

    Init   45242   46579         +         0
    Intr   46725   46788         +         0
    Intr   47076   47119         +         0
    Term   47209   47564         +         0

>3172156        /39526

   len =     1772    nex =        4

    Init   45807   46579         +         0
    Intr   46725   46788         +         0
    Intr   47076   47119         +         0
    Term   47209   47578         +         0

>3172156        /6711

   len =     1810    nex =        5

    Init   48322   48537         +         0
    Intr   48765   48805         +         0
    Intr   48909   49207         +         0
    Intr   49460   49606         +         0
    Term   49686   50127         +         0

>3172156        /120133

   len =     1781    nex =        5

    Init   48347   48537         +         0
    Intr   48765   48805         +         0
    Intr   48909   49207         +         0
    Intr   49460   49606         +         0
    Term   49686   50127         +         0

>3172156        /114146

   len =     2516    nex =        0

>3172156        /6399

   len =     2508    nex =        3

    Init   63063   63494         +         0
```

```
        Intr   63615   64173        +        0
        Term   64493   64821        +        0

  >3172156        /37537

     len =      817    nex =        4

        Term   74213   74001        -        0
        Intr   74366   74320        -        0
        Intr   74576   74517        -        0
        Init   74817   74669        -        0

  >3172156        /25408

     len =      957    nex =        4

        Term   74213   74000        -        0
        Intr   74366   74320        -        0
        Intr   74576   74517        -        0
        Init   74956   74669        -        0

  >3172156        /94230

     len =     2068    nex =        2

        Term    7070    6731        -        0
        Init    7680    7442        -        0

  >3176693        /145854

     len =     1977    nex =        2

        Init   20568   20774        +        0
        Term   20878   21151        +        0

  >3176693        /36051

     len =     1870    nex =        6

        Init   31001   31132        +        0
        Intr   31211   31309        +        0
        Intr   31406   31522        +        0
        Intr   31618   31713        +        0
        Intr   31848   31960        +        0
        Term   32037   32294        +        0

  >3176693        /28669

     len =     5624    nex =       10

        Term   36200   35812        -        0
        Intr   36523   36290        -        0
        Intr   37268   36993        -        0
        Intr   37641   37365        -        0
        Intr   38211   37913        -        0
        Intr   38769   38584        -        0
        Intr   39653   39444        -        0
        Intr   39839   39776        -        0
        Intr   40136   39990        -        0
        Init   40542   40305        -        0
```

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```
>3176693        /12338

   len =     1346   nex =        2

    Term    8005     7949         -         0
    Init    8926     8665         -         0

>3176694        /10539

   len =      704   nex =        1

    Sngl   11287    11990         +         0

>3176694        /206065

   len =     1371   nex =        3

    Term   19165    18447         -         0
    Intr   19610    19409         -         0
    Init   19817    19698         -         0

>3176694        /3965

   len =      356   nex =        1

    Sngl   29025    28693         -         0

>3176695        /39581

   len =     3411   nex =        7

    Term    8155     7742         -         0
    Intr    8402     8251         -         0
    Intr    8648     8490         -         0
    Intr    9014     8762         -         0
    Intr    9217     9101         -         0
    Intr    9356     9294         -         0
    Init   11152    10747         -         0

>3176695        /25260

   len =      897   nex =        3

    Init   26015    26131         +         0
    Intr   26251    26367         +         0
    Term   26507    26911         +         0

>3176695        /34123

   len =      215   nex =        1

    Sngl   38577    38363         -         0

>3176695        /28545

   len =      889   nex =        2

    Term   44351    43984         -         0
    Init   44872    44663         -         0
```

```
>3176695        /32366

  len =    1810   nex =       5

   Init   45733   45892       +        0
   Intr   46308   46477       +        0
   Intr   46559   46750       +        0
   Intr   46829   47178       +        0
   Term   47280   47536       +        0

>3176695        /18004

  len =     119   nex =       1

   Sngl   75443   75561       +        0

>3176701        /37338

  len =    2170   nex =       6

   Init   23063   23245       +        0
   Intr   23800   24134       +        0
   Intr   24237   24313       +        0
   Intr   24402   24462       +        0
   Intr   24573   24717       +        0
   Term   24845   25227       +        0

>3176701        /39441

  len =    1243   nex =       3

   Init   84984   85415       +        0
   Intr   85710   85873       +        0
   Term   86105   86226       +        0

>3176701        /38836

  len =     490   nex =       2

   Init   85708   85873       +        0
   Term   86105   86195       +        0

>3184270        /26825

  len =    1709   nex =       2

   Term   46892   46301       -        0
   Init   48009   47436       -        0

>3184270        /13775

  len =    2935   nex =       9

   Init   52068   52252       +        0
   Intr   52654   52722       +        0
   Intr   53313   53438       +        0
   Intr   53646   53728       +        0
   Intr   53832   53907       +        0
   Intr   53982   54158       +        0
```

```
      Intr   54241   54369        +       0
      Intr   54444   54582        +       0
      Term   54778   55002        +       0

>3184270       /12948

     len =     399    nex =       1

      Sngl   77713   77315        -       0

>3184270       /13304

     len =    2294    nex =      11

      Init   80046   80143        +       0
      Intr   80300   80371        +       0
      Intr   80466   80521        +       0
      Intr   80725   80804        +       0
      Intr   80900   80982        +       0
      Intr   81099   81176        +       0
      Intr   81270   81338        +       0
      Intr   81422   81490        +       0
      Intr   81567   81613        +       0
      Intr   81699   81789        +       0
      Term   81859   82119        +       0

>3184270       /108825

     len =    1969    nex =      10

      Init   80300   80371        +       0
      Intr   80466   80521        +       0
      Intr   80725   80804        +       0
      Intr   80900   80982        +       0
      Intr   81099   81176        +       0
      Intr   81270   81338        +       0
      Intr   81422   81490        +       0
      Intr   81567   81613        +       0
      Intr   81699   81789        +       0
      Term   81859   82035        +       0

>3193282       /13633

     len =    1915    nex =       4

      Term   16811   16369        -       0
      Intr   17513   17355        -       0
      Intr   17971   17940        -       0
      Init   18283   18065        -       0

>3193282       /20206

     len =    2050    nex =       6

      Init   35088   35203        +       0
      Intr   35751   35937        +       0
      Intr   36023   36179        +       0
      Intr   36287   36414        +       0
      Intr   36457   36643        +       0
      Term   36865   37128        +       0
```

```
>3193282        /145583

   len =      614    nex =        2

     Init   36528    36643          +         0
     Term   36865    37141          +         0

>3193282        /19681

   len =     2692    nex =       10

     Term   39121    39088          -         0
     Intr   40307    40078          -         0
     Intr   40438    40381          -         0
     Intr   40596    40547          -         0
     Intr   40783    40726          -         0
     Intr   40918    40872          -         0
     Intr   41132    40997          -         0
     Intr   41298    41224          -         0
     Intr   41506    41441          -         0
     Init   41770    41692          -         0

>3193282        /25565

   len =     1738    nex =        7

     Init   51343    51536          +         0
     Intr   51738    51895          +         0
     Intr   51986    52106          +         0
     Intr   52242    52366          +         0
     Intr   52446    52559          +         0
     Intr   52655    52822          +         0
     Term   52894    53080          +         0

>3193282        /23194

   len =      717    nex =        2

     Init   63968    64230          +         0
     Term   64331    64684          +         0

>3193282        /17524

   len =      707    nex =        1

     Sngl   68554    67848          -         0

>3193282        /19543

   len =      869    nex =        1

     Sngl   68626    67758          -         0

>3193282        /15281

   len =     1873    nex =        3

     Term   73831    73399          -         0
     Intr   74170    73932          -         0
```

```
    Init   75271   74778         -        0

>3193305       /40560

    len =    2320   nex =       6

    Term   10008    9622         -        0
    Intr   10234   10083         -        0
    Intr   10745   10346         -        0
    Intr   10952   10836         -        0
    Intr   11528   11466         -        0
    Init   11941   11625         -        0

>3193305       /29200

    len =     986   nex =       1

    Sngl   30156   29171         -        0

>3193305       /18253

    len =     670   nex =       1

    Sngl   31064   31727         +        0

>3193311       /19785

    len =    2718   nex =      10

    Init   27978   28299         +        0
    Intr   28407   28512         +        0
    Intr   28610   28670         +        0
    Intr   28784   29064         +        0
    Intr   29273   29412         +        0
    Intr   29489   29563         +        0
    Intr   29673   29763         +        0
    Intr   29932   30135         +        0
    Intr   30249   30293         +        0
    Term   30371   30695         +        0

>3193311       /4058

    len =    1211   nex =       4

    Init   30962   31056         +        0
    Intr   31136   31208         +        0
    Intr   31291   31429         +        0
    Term   31513   31699         +        0

>3193311       /12983

    len =    2068   nex =       4

    Init   57272   57337         +        0
    Intr   57712   58588         +        0
    Intr   58663   58797         +        0
    Term   58889   59339         +        0

>3193311       /32470
```

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```
    len =      610    nex =        1

     Sngl   63745   63139         -         0

>3193311         /33482

    len =     1692    nex =        7

     Term   64707   64353         -         0
     Intr   64872   64795         -         0
     Intr   65002   64961         -         0
     Intr   65233   65072         -         0
     Intr   65434   65319         -         0
     Intr   65755   65682         -         0
     Init   66044   65896         -         0

>3193311         /102945

    len =     2350    nex =        5

     Term   66929   66852         -         0
     Intr   67385   67310         -         0
     Intr   67569   67521         -         0
     Intr   68289   68130         -         0
     Init   68762   68559         -         0

>3193311         /13263

    len =     2770    nex =        6

     Term   66929   66852         -         0
     Intr   67385   67310         -         0
     Intr   67569   67521         -         0
     Intr   68289   68130         -         0
     Intr   68729   68559         -         0
     Init   69246   68838         -         0

>3193311         /21877

    len =     1450    nex =        2

     Init    8494    8695         +         0
     Term    8783    9939         +         0

>3201608         /30206

    len =      916    nex =        3

     Init   13538   13733         +         0
     Intr   13827   13875         +         0
     Term   14061   14453         +         0

>3201608         /36378

    len =      337    nex =        2

     Init   13539   13733         +         0
     Term   13827   13875         +         0

>3201608         /38967
```

```
    len =     1123    nex =        3

      Init   15910    16091         +         0
      Intr   16178    16226         +         0
      Term   16396    17032         +         0

>3201608          /142926

    len =      446    nex =        1

      Sngl   15916    16359         +         0

>3201608          /33579

    len =      430    nex =        1

      Sngl   16612    17032         +         0

>3201608          /7866

    len =     2272    nex =        4

      Init   22124    22360         +         0
      Intr   22689    22844         +         0
      Intr   23414    23562         +         0
      Term   23794    24395         +         0

>3201608          /125631

    len =      956    nex =        3

      Init   36898    37143         +         0
      Intr   37222    37407         +         0
      Term   37534    37853         +         0

>3201608          /118068

    len =      680    nex =        1

      Sngl   52418    52003         -         0

>3201608          /34360

    len =     2916    nex =       10

      Init   53417    53661         +         0
      Intr   53871    53956         +         0
      Intr   54282    54488         +         0
      Intr   54612    54717         +         0
      Intr   54802    54906         +         0
      Intr   54980    55176         +         0
      Intr   55376    55535         +         0
      Intr   55661    55756         +         0
      Intr   55848    55942         +         0
      Term   56072    56332         +         0

>3201608          /18932

    len =     3685    nex =       12
```

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```
     Term   56943   56652         -         0
     Intr   57107   57006         -         0
     Intr   57319   57236         -         0
     Intr   57540   57445         -         0
     Intr   57666   57616         -         0
     Intr   57901   57761         -         0
     Intr   58146   58090         -         0
     Intr   58297   58223         -         0
     Intr   58658   58386         -         0
     Intr   58800   58741         -         0
     Intr   59106   59014         -         0
     Init   60336   60218         -         0

>3201608        /36479

    len =     3770    nex =       11

     Term   57107   57006         -         0
     Intr   57319   57236         -         0
     Intr   57540   57445         -         0
     Intr   57666   57616         -         0
     Intr   57901   57761         -         0
     Intr   58146   58090         -         0
     Intr   58297   58223         -         0
     Intr   58658   58386         -         0
     Intr   58800   58741         -         0
     Intr   59106   59014         -         0
     Init   60381   60218         -         0

>3201608        /35095

    len =     2050    nex =        9

     Term   83717   83480         -         0
     Intr   83885   83817         -         0
     Intr   84083   83991         -         0
     Intr   84260   84169         -         0
     Intr   84508   84428         -         0
     Intr   84761   84666         -         0
     Intr   84937   84841         -         0
     Intr   85083   85034         -         0
     Init   85526   85162         -         0

>3212102        /36315

    len =     1724    nex =        8

     Init   20146   20243         +         0
     Intr   20513   20640         +         0
     Intr   20801   20899         +         0
     Intr   20991   21149         +         0
     Intr   21233   21319         +         0
     Intr   21394   21485         +         0
     Intr   21563   21637         +         0
     Term   21735   21869         +         0

>3212102        /124895

    len =      930    nex =        2
```

```
      Term    23160    22726          -         0
      Init    23655    23565          -         0

    >3212102        /7088

     len =     1376    nex =         2

      Term    23160    22971          -         0
      Init    24346    23565          -         0

    >3212102        /42528

     len =     1189    nex =         2

      Init    54373    54915          +         0
      Term    55009    55561          +         0

    >3212102        /34748

     len =      631    nex =         1

      Sngl    71980    71350          -         0

    >3212102        /25849

     len =      437    nex =         1

      Sngl    71980    71544          -         0

    >3212846        /30161

     len =     1396    nex =         6

      Term   100365   100258          -         0
      Intr   100551   100455          -         0
      Intr   100842   100748          -         0
      Intr   101023   100927          -         0
      Intr   101352   101107          -         0
      Init   101653   101420          -         0

    >3212846        /24370

     len =     2551    nex =         8

      Term    14963    14742          -         0
      Intr    15363    15285          -         0
      Intr    15522    15476          -         0
      Intr    15716    15617          -         0
      Intr    15974    15874          -         0
      Intr    16145    16059          -         0
      Intr    16807    16677          -         0
      Init    17292    17119          -         0

    >3212846        /36599

     len =      598    nex =         2

      Term    16807    16695          -         0
      Init    17292    17119          -         0
```

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```
>3212846        /106170

   len =     1639    nex =        6

    Term    19624    19275          -          0
    Intr    19851    19773          -          0
    Intr    20034    19988          -          0
    Intr    20229    20130          -          0
    Intr    20408    20308          -          0
    Init    20607    20521          -          0

>3212846        /38206

   len =     2304    nex =        6

    Term    28397    28158          -          0
    Intr    28537    28479          -          0
    Intr    28784    28635          -          0
    Intr    28981    28865          -          0
    Intr    29343    29149          -          0
    Init    30461    30043          -          0

>3212846        /482

   len =     1849    nex =        7

    Term    36128    35534          -          0
    Intr    36364    36203          -          0
    Intr    36609    36541          -          0
    Intr    36747    36700          -          0
    Intr    36942    36840          -          0
    Intr    37120    37041          -          0
    Init    37271    37200          -          0

>3212846        /36111

   len =     1817    nex =        8

    Term    52320    52252          -          0
    Intr    52529    52442          -          0
    Intr    52740    52631          -          0
    Intr    52934    52833          -          0
    Intr    53111    53037          -          0
    Intr    53340    53207          -          0
    Intr    53778    53541          -          0
    Init    54068    53940          -          0

>3212846        /10293

   len =     2263    nex =        8

    Term    52320    52036          -          0
    Intr    52529    52442          -          0
    Intr    52740    52631          -          0
    Intr    52934    52833          -          0
    Intr    53111    53037          -          0
    Intr    53340    53207          -          0
    Intr    53778    53541          -          0
    Init    54153    53940          -          0
```

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```
>3212846       /26137

  len =    1882   nex =       8

   Init   54528   54721        +        0
   Intr   54809   54924        +        0
   Intr   55061   55156        +        0
   Intr   55233   55328        +        0
   Intr   55472   55550        +        0
   Intr   55791   55837        +        0
   Intr   55936   55995        +        0
   Term   56146   56409        +        0

>3212846       /23761

  len =    1826   nex =       1

   Sngl   58250   58411        +        0

>3212846       /2450

  len =    1830   nex =       2

   Init   57184   58160        +        0
   Term   58250   58415        +        0

>3212846       /24003

  len =    2050   nex =       3

   Init   58759   58825        +        0
   Intr   59065   59223        +        0
   Term   60088   60794        +        0

>3212846       /23722

  len =    2058   nex =       3

   Init   58759   58825        +        0
   Intr   59065   59223        +        0
   Term   60088   60804        +        0

>3212846       /29536

  len =    3420   nex =      13

   Init   71959   72157        +        0
   Intr   72507   72569        +        0
   Intr   72658   72741        +        0
   Intr   72831   72938        +        0
   Intr   73127   73299        +        0
   Intr   73393   73504        +        0
   Intr   73614   73694        +        0
   Intr   73795   73857        +        0
   Intr   73975   74076        +        0
   Intr   74249   74373        +        0
   Intr   74619   74829        +        0
   Intr   74912   74986        +        0
   Term   75078   75378        +        0
```

```
>3212846        /4611

  len =    2878    nex =      11

   Init   72071   72157       +        0
   Intr   72507   72569       +        0
   Intr   72658   72741       +        0
   Intr   72831   72938       +        0
   Intr   73127   73299       +        0
   Intr   73393   73504       +        0
   Intr   73614   73694       +        0
   Intr   73795   73857       +        0
   Intr   73975   74076       +        0
   Intr   74249   74373       +        0
   Term   74619   74829       +        0

>3212846        /36813

  len =    1667    nex =       5

   Term   79812   79309       -        0
   Intr   79978   79899       -        0
   Intr   80211   80154       -        0
   Intr   80392   80295       -        0
   Init   80975   80496       -        0

>3212846        /17409

  len =    1733    nex =       5

   Term   79812   79361       -        0
   Intr   79978   79899       -        0
   Intr   80211   80154       -        0
   Intr   80392   80295       -        0
   Init   81093   80496       -        0

>3212846        /30978

  len =    1706    nex =       4

   Term   79812   79388       -        0
   Intr   80211   79899       -        0
   Intr   80392   80295       -        0
   Init   81093   80496       -        0

>3212846        /6950

  len =    1390    nex =       1

   Sngl   83877   85266       +        0

>3212846        /17908

  len =    2271    nex =       8

   Init   93341   93451       +        0
   Intr   93759   93932       +        0
   Intr   94012   94112       +        0
   Intr   94210   94321       +        0
```

```
        Intr    94487    94567         +          0
        Intr    94665    94842         +          0
        Intr    94936    95270         +          0
        Term    95369    95611         +          0

   >3228389         /117479

       len =      1270    nex =         2

        Init    25417    26084         +          0
        Term    26445    26682         +          0

   >3228389         /7227

       len =      1347    nex =         2

        Init    25417    26084         +          0
        Term    26445    26763         +          0

   >3228389         /15453

       len =      1292    nex =         2

        Term    42250    42036         -          0
        Init    42669    42513         -          0

   >3228389         /2617

       len =      1296    nex =         2

        Term    42250    42033         -          0
        Init    42669    42513         -          0

   >3228389         /42666

       len =      1771    nex =         4

        Init    45914    46685         +          0
        Intr    46846    46967         +          0
        Intr    47100    47351         +          0
        Term    47455    47684         +          0

   >3228389         /39286

       len =      1930    nex =         5

        Term    55070    54825         -          0
        Intr    55329    55246         -          0
        Intr    55816    55703         -          0
        Intr    56104    56001         -          0
        Init    56745    56456         -          0

   >3228389         /18771

       len =       320    nex =         1

        Sngl    57320    57001         -          0

   >3228389         /749
```

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```
     len =     1712    nex =        7

      Init   59020    59117          +         0
      Intr   59214    59250          +         0
      Intr   59369    59409          +         0
      Intr   59889    60119          +         0
      Intr   60204    60306          +         0
      Intr   60397    60464          +         0
      Term   60563    60731          +         0

>3228389        /34212

     len =     1113    nex =        2

      Term   71736    71610          -         0
      Init   72722    72553          -         0

>3228389        /22723

     len =     1056    nex =        1

      Sngl   72763    72553          -         0

>3228389        /3279

     len =     1656    nex =        6

      Init   76223    76387          +         0
      Intr   76478    76588          +         0
      Intr   76756    76848          +         0
      Intr   77250    77399          +         0
      Intr   77484    77542          +         0
      Term   77636    77878          +         0

>3228389        /35408

     len =     1760    nex =        2

      Init   88411    88892          +         0
      Term   89156    90170          +         0

>3236234        /42701

     len =     2562    nex =        5

      Init   14714    14911          +         0
      Intr   15858    15964          +         0
      Intr   16057    16189          +         0
      Intr   16311    16602          +         0
      Term   16755    17275          +         0

>3236234        /116968

     len =      826    nex =        1

      Sngl   20794    21619          +         0

>3236234        /18641

     len =      814    nex =        1
```

```
    Sngl    21539    20726          -        0

>3236234         /34743

    len =     1033    nex =        3

    Term    30515    30312          -        0
    Intr    30858    30659          -        0
    Init    31344    31168          -        0

>3236234         /23114

    len =     1095    nex =        3

    Term    30515    30314          -        0
    Intr    30858    30659          -        0
    Init    31275    31168          -        0

>3236234         /19581

    len =     2170    nex =        4

    Init    35817    35886          +        0
    Intr    36023    36416          +        0
    Intr    36938    37130          +        0
    Term    37242    37510          +        0

>3236234         /38101

    len =     1484    nex =        4

    Init    35822    35886          +        0
    Intr    36023    36416          +        0
    Intr    36478    37130          +        0
    Term    37242    37305          +        0

>3236234         /25785

    len =     1487    nex =        4

    Init    35822    35886          +        0
    Intr    36023    36416          +        0
    Intr    36938    37130          +        0
    Term    37242    37308          +        0

>3236234         /13797

    len =     1695    nex =        7

    Term    51666    51431          -        0
    Intr    51905    51782          -        0
    Intr    52049    51988          -        0
    Intr    52228    52131          -        0
    Intr    52342    52306          -        0
    Intr    52698    52427          -        0
    Init    53125    52779          -        0

>3236234         /28529
```

```
    len =       529    nex =        1

      Sngl   87056   87584         +        0

>3236479        /10950

    len =      1705    nex =        2

      Init   28178   28532         +        0
      Term   29443   29882         +        0

>3236479        /30441

    len =       285    nex =        1

      Sngl   28227   28511         +        0

>3236479        /143197

    len =       439    nex =        1

      Sngl   38868   39306         +        0

>3236479        /32070

    len =      2470    nex =        4

      Term   89296   89181         -        0
      Intr   89764   89651         -        0
      Intr   89981   89841         -        0
      Init   91120   90101         -        0

>3241916        /143222

    len =      1330    nex =        6

      Term   17392   17118         -        0
      Intr   17601   17497         -        0
      Intr   17779   17689         -        0
      Intr   17948   17886         -        0
      Intr   18263   18214         -        0
      Init   18446   18359         -        0

>3241916        /7891

    len =      1510    nex =        2

      Init   19560   19940         +        0
      Term   20202   21066         +        0

>3241916        /105027

    len =       167    nex =        1

      Sngl   42357   42523         +        0

>3241916        /2036

    len =      1660    nex =        9
```

```
     Term   42888   42671          -         0
     Intr   43123   42976          -         0
     Intr   43297   43247          -         0
     Intr   43511   43397          -         0
     Intr   43721   43603          -         0
     Intr   43846   43802          -         0
     Intr   43979   43940          -         0
     Intr   44199   44093          -         0
     Init   44330   44290          -         0

>3241916        /12974

    len =     1882    nex =        5

     Term   52467   52276          -         0
     Intr   52920   52744          -         0
     Intr   53121   53006          -         0
     Intr   53292   53235          -         0
     Init   53637   53395          -         0

>3241917        /148899

    len =     1424    nex =        2

     Term   12645   12184          -         0
     Init   13607   13335          -         0

>3241917        /3896

    len =     1486    nex =        2

     Term   12645   12218          -         0
     Init   13703   13335          -         0

>3241917        /152864

    len =      319    nex =        1

     Sngl   13703   13385          -         0

>3241917        /119748

    len =      794    nex =        2

     Term   28884   28539          -         0
     Init   29332   28954          -         0

>3241917        /23920

    len =     1487    nex =        4

     Init   37712   38242          +         0
     Intr   38331   38575          +         0
     Intr   38659   38777          +         0
     Term   38871   39198          +         0

>3241917        /148141

    len =     1491    nex =        5
```

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```
      Init   44373   44517        +        0
      Intr   44592   44617        +        0
      Intr   44716   44805        +        0
      Intr   44885   44973        +        0
      Term   45057   45296        +        0

>3241917       /37636

     len =     939   nex =       5

      Init   44355   44517        +        0
      Intr   44592   44617        +        0
      Intr   44716   44805        +        0
      Intr   44885   44973        +        0
      Term   45057   45293        +        0

>3241917       /34484

     len =     490   nex =       1

      Sngl   51169   51654        +        0

>3241917       /30313

     len =    1235   nex =       4

      Init   57202   57315        +        0
      Intr   57401   57469        +        0
      Intr   57768   57870        +        0
      Term   58209   58436        +        0

>3241920       /19080

     len =    1218   nex =       1

      Sngl   45667   46884        +        0

>3241921       /42897

     len =     475   nex =       1

      Sngl   26675   26201        -        0

>3241921       /33053

     len =     555   nex =       1

      Sngl   35941   36495        +        0

>3241922       /29124

     len =    1276   nex =       4

      Init   18356   18395        +        0
      Intr   18580   18665        +        0
      Intr   18750   18873        +        0
      Term   19391   19631        +        0

>3241922       /34761
```

```
   len =      420    nex =        1

    Sngl      288     707          +        0

>3241922        /13557

   len =     1016    nex =        4

    Init    47961   48011          +        0
    Intr    48229   48408          +        0
    Intr    48587   48661          +        0
    Term    48747   48972          +        0

>3241922        /29542

   len =     1845    nex =        4

    Term    49432   49038          -        0
    Intr    49649   49523          -        0
    Intr    50374   50138          -        0
    Init    50882   50532          -        0

>3241922        /100927

   len =      391    nex =        1

    Sngl    58311   58701          +        0

>3241922        /34244

   len =      166    nex =        1

    Sngl    84414   84579          +        0

>3241923        /40576

   len =      765    nex =        1

    Sngl    16439   17203          +        0

>3241923        /8215

   len =     1690    nex =        8

    Term    21000   20843          -        0
    Intr    21226   21110          -        0
    Intr    21370   21308          -        0
    Intr    21576   21479          -        0
    Intr    21730   21665          -        0
    Intr    22067   21967          -        0
    Intr    22235   22163          -        0
    Init    22526   22444          -        0

>3241923        /11699

   len =      600    nex =        1

    Sngl    24791   24991          +        0

>3241923        /7822
```

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```
      len =      595    nex =        3

       Term   26064    25987         -         0
       Intr   26320    26160         -         0
       Init   26559    26419         -         0

  >3241923        /36757

      len =     1390    nex =        2

       Term   26064    25685         -         0
       Init   26320    26160         -         0

  >3241923        /20036

      len =      734    nex =        2

       Term   29311    28952         -         0
       Init   29685    29399         -         0

  >3241923        /20973

      len =     1537    nex =        3

       Init   33738    33851         +         0
       Intr   34586    34851         +         0
       Term   34945    35274         +         0

  >3241923        /11691

      len =     1472    nex =        2

       Init   36556    36821         +         0
       Term   36913    37365         +         0

  >3241923        /9208

      len =      510    nex =        2

       Init   36555    36821         +         0
       Term   36913    37064         +         0

  >3241923        /20822

      len =      715    nex =        1

       Sngl   40595    41309         +         0

  >3241923        /114182

      len =      399    nex =        1

       Sngl   40736    41127         +         0

  >3241923        /27649

      len =     1630    nex =        5

       Init   53569    53872         +         0
```

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```
      Intr    54069    54212          +        0
      Intr    54361    54429          +        0
      Intr    54523    54701          +        0
      Term    54800    55196          +        0

>3241923        /26907

    len =     1617    nex =       5

      Init    53602    53872          +        0
      Intr    54069    54212          +        0
      Intr    54361    54429          +        0
      Intr    54523    54701          +        0
      Term    54800    55218          +        0

>3241923        /31173

    len =      440    nex =       1

      Sngl    60193    59754          -        0

>3241923        /4349

    len =     1995    nex =       6

      Term     8413     7946          -        0
      Intr     8649     8527          -        0
      Intr     8936     8739          -        0
      Intr     9218     9120          -        0
      Intr     9456     9304          -        0
      Init     9940     9737          -        0

>3241924        /4012

    len =      407    nex =       0

>3241924        /2034

    len =      574    nex =       1

      Sngl     2980     3553          +        0

>3241924        /41458

    len =     2372    nex =      10

      Term    32689    32396          -        0
      Intr    32948    32773          -        0
      Intr    33091    33036          -        0
      Intr    33270    33226          -        0
      Intr    33426    33360          -        0
      Intr    33636    33525          -        0
      Intr    33778    33734          -        0
      Intr    33948    33876          -        0
      Intr    34113    34039          -        0
      Init    34286    34212          -        0

>3241924        /38213

    len =     1734    nex =       5
```

```
        Term   36042   35649         -         0
        Intr   36432   36137         -         0
        Intr   36674   36516         -         0
        Intr   37042   36742         -         0
        Init   37382   37126         -         0

   >3241924        /31303

       len =     1072    nex =        3

        Init   45856   45947         +         0
        Intr   46181   46247         +         0
        Term   46336   46715         +         0

   >3241924        /14377

       len =      768    nex =        3

        Term   51633   51312         -         0
        Intr   51804   51711         -         0
        Init   52079   51889         -         0

   >3241924        /38528

       len =     1902    nex =        6

        Term   51633   51293         -         0
        Intr   51804   51711         -         0
        Intr   52142   51889         -         0
        Intr   52355   52232         -         0
        Intr   52541   52448         -         0
        Init   53194   53036         -         0

   >3241924        /7358

       len =      490    nex =        1

        Sngl   58044   58525         +         0

   >3241924        /106913

       len =     1215    nex =        2

        Init   77459   77620         +         0
        Term   78380   78673         +         0

   >3241925        /10394

       len =     2076    nex =        6

        Term   49380   48998         -         0
        Intr   49603   49472         -         0
        Intr   49732   49678         -         0
        Intr   50653   50482         -         0
        Intr   50890   50743         -         0
        Init   51073   50979         -         0

   >3241926        /25388
```

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```
     len =      944    nex =        2

      Init   12191    12401          +         0
      Term   12491    12805          +         0

>3241926        /41509

     len =     1870    nex =        4

      Init   13521    13588          +         0
      Intr   13687    14214          +         0
      Intr   14299    14486          +         0
      Term   14580    14889          +         0

>3241926        /13875

     len =     1717    nex =        5

      Init   13173    13425          +         0
      Intr   13521    13588          +         0
      Intr   13687    14214          +         0
      Intr   14299    14486          +         0
      Term   14580    14889          +         0

>3241926        /18612

     len =     2833    nex =        7

      Term   15300    14853          -         0
      Intr   15460    15363          -         0
      Intr   15616    15557          -         0
      Intr   15814    15695          -         0
      Intr   16062    15916          -         0
      Intr   16256    16155          -         0
      Init   17421    16839          -         0

>3241926        /8268

     len =     1345    nex =        6

      Init    6075     6204          +         0
      Intr    6527     6560          +         0
      Intr    6653     6825          +         0
      Intr    6914     6965          +         0
      Intr    7061     7128          +         0
      Term    7215     7419          +         0

>3241926        /206563

     len =     1570    nex =        5

      Term   74328    73909          -         0
      Intr   74617    74464          -         0
      Intr   74837    74697          -         0
      Intr   74994    74923          -         0
      Init   75192    75080          -         0

>3241927        /4309

     len =      757    nex =        0
```

```
>3241927        /32995

   len =    2052   nex =        0

>3241939        /27423

   len =    1306   nex =        3

     Init   2469    2639        +        0
     Intr   2957    3312        +        0
     Term   3400    3774        +        0

>3241939        /31388

   len =     970   nex =        2

     Init   2957    3312        +        0
     Term   3400    3649        +        0

>3241939        /41130

   len =    1254   nex =        3

     Term  28798   28404        -        0
     Intr  29363   29256        -        0
     Init  29657   29438        -        0

>3242700        /120446

   len =     358   nex =        1

     Sngl  37693   38050        +        0

>3242700        /25463

   len =    1390   nex =        2

     Term  93971   93636        -        0
     Init  95019   94055        -        0

>3242700        /26006

   len =    1391   nex =        3

     Term  93971   93636        -        0
     Intr  94189   94055        -        0
     Init  95026   94632        -        0

>3242970        /34126

   len =    1718   nex =        5

     Init  61864   62009        +        0
     Intr  62392   62498        +        0
     Intr  62588   62788        +        0
     Intr  63185   63249        +        0
     Term  63334   63581        +        0

>3242970        /99825
```

```
     len =     1815    nex =        5

      Init   61865    62009          +         0
      Intr   62392    62498          +         0
      Intr   62588    62788          +         0
      Intr   63185    63249          +         0
      Term   63334    63679          +         0

 >3243214         /24559

     len =     1330    nex =        3

      Term   22739    22559          -         0
      Intr   23241    23208          -         0
      Init   23516    23386          -         0

 >3249094         /9228

     len =      951    nex =        1

      Sngl   12849    11899          -         0

 >3249094         /37124

     len =     1184    nex =        1

      Sngl   17368    16185          -         0

 >3249094         /27210

     len =     3696    nex =       13

      Init   25520    25687          +         0
      Intr   26293    26400          +         0
      Intr   26495    26687          +         0
      Intr   26785    27043          +         0
      Intr   27217    27321          +         0
      Intr   27408    27494          +         0
      Intr   27577    27634          +         0
      Intr   28142    28209          +         0
      Intr   28295    28390          +         0
      Intr   28484    28531          +         0
      Intr   28693    28722          +         0
      Intr   28807    28923          +         0
      Term   29021    29215          +         0

 >3249094         /10388

     len =      587    nex =        1

      Sngl   30050    30636          +         0

 >3249094         /1854

     len =      792    nex =        3

      Term   36628    36312          -         0
      Intr   36865    36734          -         0
      Init   37103    36974          -         0
```

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```
>3249094        /31022

   len =     1314    nex =        3

    Init   51260    51655        +        0
    Intr   51724    51842        +        0
    Term   51917    52573        +        0

>3249094        /110171

   len =     2770    nex =        8

    Term   53358    53234        -        0
    Intr   53539    53476        -        0
    Intr   53856    53789        -        0
    Intr   54097    53939        -        0
    Intr   54429    54377        -        0
    Intr   54692    54595        -        0
    Intr   54861    54794        -        0
    Init   55039    54958        -        0

>3249094        /7559

   len =     2785    nex =       11

    Init   56298    56517        +        0
    Intr   56641    56706        +        0
    Intr   56793    56867        +        0
    Intr   56947    57049        +        0
    Intr   57135    57181        +        0
    Intr   57289    57346        +        0
    Intr   57470    57519        +        0
    Intr   57663    57719        +        0
    Intr   57793    57905        +        0
    Intr   58003    58080        +        0
    Term   59023    59082        +        0

>3250673        /3164

   len =      734    nex =        1

    Sngl   17027    17760        +        0

>3250673        /17437

   len =     1613    nex =        3

    Init   83877    84182        +        0
    Intr   84570    84691        +        0
    Term   84930    85489        +        0

>3250673        /34882

   len =     1175    nex =        3

    Term   94732    94604        -        0
    Intr   95043    94819        -        0
    Init   95309    95132        -        0
```

```
>3252804        /40603

   len =     4015    nex =       10

    Term    17729    17324          -          0
    Intr    18030    17844          -          0
    Intr    18354    18243          -          0
    Intr    18716    18462          -          0
    Intr    19017    18946          -          0
    Intr    19896    19802          -          0
    Intr    20112    19995          -          0
    Intr    20703    20628          -          0
    Intr    21077    20991          -          0
    Init    21338    21278          -          0

>3252804        /14216

   len =     1608    nex =        7

    Term    72843    72609          -          0
    Intr    73012    72966          -          0
    Intr    73182    73098          -          0
    Intr    73389    73271          -          0
    Intr    73624    73475          -          0
    Intr    73849    73709          -          0
    Init    74216    73929          -          0

>3269280        /15604

   len =      971    nex =        2

    Term    13730    13454          -          0
    Init    14424    14146          -          0

>3269280        /927

   len =     1073    nex =        2

    Term    13730    13356          -          0
    Init    14428    14146          -          0

>3269280        /2944

   len =     1694    nex =        2

    Init    15972    16829          +          0
    Term    17015    17665          +          0

>3269280        /13072

   len =      670    nex =        1

    Sngl    40249    39588          -          0

>3269280        /17584

   len =      594    nex =        1

    Sngl    40252    39659          -          0
```

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```
>3269280        /10313

   len =     2310    nex =        2

    Term   54740    54478         -         0
    Init   56787    55952         -         0

>3269280        /35749

   len =     1969    nex =        2

    Term   67347    66835         -         0
    Init   68803    68154         -         0

>3269280        /39662

   len =     1890    nex =        5

    Init   77267    77439         +         0
    Intr   77520    77574         +         0
    Intr   78008    78078         +         0
    Intr   78194    78302         +         0
    Term   78818    79156         +         0

>3269280        /40387

   len =      893    nex =        2

    Init   81954    82059         +         0
    Term   82190    82846         +         0

>3281847        /18361

   len =      476    nex =        2

    Term   10602    10258         -         0
    Init   10733    10682         -         0

>3281847        /25793

   len =     2313    nex =        6

    Init   31587    31715         +         0
    Intr   31812    31876         +         0
    Intr   32278    32340         +         0
    Intr   32526    32577         +         0
    Intr   32672    32722         +         0
    Term   33024    33293         +         0

>3281847        /34408

   len =     1487    nex =        5

    Init   39703    39769         +         0
    Intr   40039    40096         +         0
    Intr   40195    40303         +         0
    Intr   40413    40685         +         0
    Term   40780    41189         +         0

>3281847        /33868
```

```
      len =     1007    nex =        3

       Init   40194    40303         +         0
       Intr   40413    40685         +         0
       Term   40780    41200         +         0

 >3281847         /40692

      len =     1740    nex =        4

       Term     2811     2385        -         0
       Intr     3511     3125        -         0
       Intr     3838     3776        -         0
       Init     4124     3963        -         0

 >3281847         /96391

      len =      771    nex =        3

       Term   79299    78987         -         0
       Intr   79557    79423         -         0
       Init   79757    79637         -         0

 >3281847         /25581

      len =     1595    nex =        5

       Term   79299    79054         -         0
       Intr   79557    79423         -         0
       Intr   79889    79637         -         0
       Intr   80152    80064         -         0
       Init   80356    80256         -         0

 >3282170         /19175

      len =     1398    nex =        5

       Init  110143   110193         +         0
       Intr  110394   110449         +         0
       Intr  110536   110588         +         0
       Intr  110832   110950         +         0
       Term  111100   111270         +         0

 >3282170         /2843

      len =      656    nex =        2

       Term  112059   111629         -         0
       Init  112284   112232         -         0

 >3282170         /16387

      len =     1588    nex =        1

       Sngl   43553    41966         -         0

 >3282170         /39661

      len =     2445    nex =        7
      Term   59522   59409        -        0
      Intr   59658   59614        -        0
      Intr   59899   59756        -        0
      Intr   60368   60000        -        0
      Intr   61028   60447        -        0
      Intr   61250   61122        -        0
      Init   61853   61594        -        0

>3282170        /30230

    len =     870   nex =       1

      Sngl   70042   70911        +        0

>3282170        /32125

    len =    1584   nex =       1

      Sngl   84969   86552        +        0

>3282170        /2939

    len =     310   nex =       1

      Sngl   86228   86537        +        0

>3282170        /25426

    len =     458   nex =       1

      Sngl   88205   88662        +        0

>3292807        /42959

    len =    1450   nex =       2

      Term   18522   17812        -        0
      Init   19126   18607        -        0

>3292807        /13264

    len =    2439   nex =       9

      Init   20030   20399        +        0
      Intr   20826   20938        +        0
      Intr   21037   21133        +        0
      Intr   21226   21326        +        0
      Intr   21422   21478        +        0
      Intr   21563   21639        +        0
      Intr   21733   21895        +        0
      Intr   21975   22072        +        0
      Term   22302   22468        +        0

>3292807        /21391

    len =    2384   nex =       9

      Init   20085   20399        +        0
      Intr   20826   20938        +        0
    Intr   21037   21133        +       0
    Intr   21226   21326        +       0
    Intr   21422   21478        +       0
    Intr   21563   21639        +       0
    Intr   21733   21895        +       0
    Intr   21975   22072        +       0
    Term   22302   22468        +       0

>3292807        /95912

   len =     347   nex =       1

    Sngl   22302   22507        +       0

>3292807        /29391

   len =    1588   nex =       4

    Term   30143   29734        -       0
    Intr   30387   30220        -       0
    Intr   30755   30582        -       0
    Init   31321   30873        -       0

>3292807        /8228

   len =     625   nex =       2

    Term   73592   73242        -       0
    Init   73866   73675        -       0

>3292807        /25628

   len =    2590   nex =       6

    Term   73592   73176        -       0
    Intr   73782   73675        -       0
    Intr   74304   74203        -       0
    Intr   74936   74735        -       0
    Intr   75046   75018        -       0
    Init   75763   75408        -       0

>3293581        /13181

   len =    1475   nex =       3

    Term   69408   68947        -       0
    Intr   69691   69496        -       0
    Init   70421   70034        -       0

>3293581        /1461

   len =    1570   nex =       3

    Term   69408   68922        -       0
    Intr   69691   69496        -       0
    Init   70489   70034        -       0

>3293581        /39258

   len =    1590   nex =       3
```

```
      Term   69408   68907         -        0
      Intr   69691   69496         -        0
      Init   70496   70034         -        0

>3293582        /97031

     len =     390   nex =       1

      Sngl   22292   21903         -        0

>3293582        /14258

     len =    3176   nex =       7

      Term   50181   49892         -        0
      Intr   50364   50280         -        0
      Intr   51074   50997         -        0
      Intr   51309   51162         -        0
      Intr   51674   51515         -        0
      Intr   52551   52516         -        0
      Init   53067   53029         -        0

>3293583        /111377

     len =    1211   nex =       1

      Sngl   46281   46069         -        0

>3293583        /1463

     len =    1510   nex =       4

      Term   51608   51036         -        0
      Intr   51864   51769         -        0
      Intr   52214   51938         -        0
      Init   52536   52350         -        0

>3293583        /112432

     len =    1849   nex =       3

      Term   55125   55072         -        0
      Intr   55543   55514         -        0
      Init   55756   55620         -        0

>3297806        /24629

     len =     971   nex =       2

      Term   18135   18036         -        0
      Init   18729   18586         -        0

>3297806        /21867

     len =    1979   nex =       7

      Term   41785   41666         -        0
      Intr   41937   41872         -        0
      Intr   42102   42007         -        0
```

```
                                              1152
         Intr    42340    42238        -         0
         Intr    42580    42423        -         0
         Intr    42933    42811        -         0
         Init    43304    43034        -         0

    >3297806        /2306

        len =     1972    nex =        7

         Term    41785    41666        -         0
         Intr    41937    41872        -         0
         Intr    42102    42007        -         0
         Intr    42340    42238        -         0
         Intr    42580    42423        -         0
         Intr    42933    42811        -         0
         Init    43304    43034        -         0

    >3297806        /40123

        len =     2093    nex =        4

         Init    45275    45667        +         0
         Intr    45924    46300        +         0
         Intr    46640    46876        +         0
         Term    46967    47367        +         0

    >3297806        /24360

        len =      988    nex =        5

         Init    47770    47885        +         0
         Intr    47971    47992        +         0
         Intr    48108    48213        +         0
         Intr    48295    48402        +         0
         Term    48496    48757        +         0

    >3297806        /14385

        len =     1719    nex =        4

         Init    50596    50900        +         0
         Intr    51195    51391        +         0
         Intr    51826    51877        +         0
         Term    51988    52314        +         0

    >3297806        /8161

        len =     2257    nex =        4

         Term    67615    67335        -         0
         Intr    67811    67699        -         0
         Intr    68621    68463        -         0
         Init    69591    69324        -         0

    >3297806        /9341

        len =      790    nex =        2

         Term    72925    72662        -         0
         Init    73446    73018        -         0
```

```
>3297806        /6896

   len =     2658    nex =       8

    Term   83301    82800          -         0
    Intr   83518    83381          -         0
    Intr   83942    83742          -         0
    Intr   84248    84171          -         0
    Intr   84531    84352          -         0
    Intr   84770    84621          -         0
    Intr   85083    84883          -         0
    Init   85457    85176          -         0

>3298532        /18983

   len =     1557    nex =       1

    Sngl   10434     8878          -         0

>3298532        /2114

   len =     1830    nex =       5

    Term   26390    26016          -         0
    Intr   26666    26475          -         0
    Intr   26876    26747          -         0
    Intr   27100    26958          -         0
    Init   27845    27756          -         0

>3298532        /630

   len =     2088    nex =       4

    Init   45179    45777          +         0
    Intr   45865    46206          +         0
    Intr   46287    46361          +         0
    Term   46938    47266          +         0

>3298532        /16625

   len =     1601    nex =       3

    Init   48070    48390          +         0
    Intr   49014    49037          +         0
    Term   49150    49670          +         0

>3298532        /28303

   len =     2115    nex =       7

    Term   54352    53910          -         0
    Intr   54548    54474          -         0
    Intr   54758    54684          -         0
    Intr   54914    54849          -         0
    Intr   55095    55019          -         0
    Intr   55479    55310          -         0
    Init   56024    55735          -         0

>3298532        /41121
```

```
     len =      454    nex =        1

      Sngl   64301   64754          +         0

>3299824        /29869

     len =     2330    nex =        5

      Term   16577   16462          -         0
      Intr   17378   17142          -         0
      Intr   17531   17470          -         0
      Intr   18024   17899          -         0
      Init   18270   18221          -         0

>3299824        /20753

     len =     1870    nex =        5

      Init   18439   18529          +         0
      Intr   18632   18674          +         0
      Intr   19090   19131          +         0
      Intr   19213   19441          +         0
      Term   19805   20301          +         0

>3299824        /15442

     len =      617    nex =        2

      Init   25851   25868          +         0
      Term   25952   26467          +         0

>3299824        /711

     len =     1732    nex =        2

      Init   35976   36226          +         0
      Term   37044   37707          +         0

>3299824        /113135

     len =     1721    nex =        2

      Init   35987   36226          +         0
      Term   37044   37707          +         0

>3299824        /17748

     len =     1376    nex =        4

      Init   48724   48965          +         0
      Intr   49089   49189          +         0
      Intr   49289   49482          +         0
      Term   49567   50099          +         0

>3299824        /93014

     len =      960    nex =        3

      Init   73060   73121          +         0
```

```
       Intr    73213    73463          +        0
       Term    73906    74019          +        0

    >3299824        /111207

      len =     1604    nex =        3

       Init    97176    97361          +        0
       Intr    97704    97837          +        0
       Term    97933    98779          +        0

    >3299824        /37727

      len =     1410    nex =        2

       Init    99110    99786          +        0
       Term    99950   100519          +        0

    >3309259        /40330

      len =     1966    nex =        3

       Init    85348    85495          +        0
       Intr    85653    85944          +        0
       Term    86040    87313          +        0

    >3309259        /27711

      len =     1630    nex =        4

       Term    89111    88858          -        0
       Intr    89328    89210          -        0
       Intr    89667    89615          -        0
       Init    89802    89747          -        0

    >3309259        /119712

      len =     1705    nex =        4

       Term    89111    88841          -        0
       Intr    89328    89210          -        0
       Intr    89667    89615          -        0
       Init    89802    89747          -        0
    >3309259        /118778

      len =      987    nex =        1

       Sngl    93145    92159          -        0

    >3309276        /7149

      len =     1455    nex =        6

       Term    49451    49232          -        0
       Intr    49635    49550          -        0
       Intr    49804    49734          -        0
       Intr    50152    50051          -        0
       Intr    50352    50254          -        0
       Init    50686    50577          -        0
```

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```
>3319339       /6836

   len =     1231   nex =       1

    Sngl     1451    2681        +       0

>3319339       /34540

   len =     1813   nex =       5

    Init    45077   45206        +       0
    Intr    45305   45474        +       0
    Intr    45606   45885        +       0
    Intr    45999   46165        +       0
    Term    46268   46560        +       0

>3319339       /32443

   len =     2033   nex =       5

    Term     3121    2602        -       0
    Intr     3336    3204        -       0
    Intr     3716    3488        -       0
    Intr     4312    4280        -       0
    Init     4634    4405        -       0

>3319339       /1360

   len =     1870   nex =       7

    Init    52990   53088        +       0
    Intr    53171   53228        +       0
    Intr    53336   53462        +       0
    Intr    53548   53717        +       0
    Intr    53870   54149        +       0
    Intr    54243   54409        +       0
    Term    54518   54850        +       0

>3319339       /27916

   len =     1832   nex =       7

    Init    52992   53088        +       0
    Intr    53171   53228        +       0
    Intr    53336   53462        +       0
    Intr    53548   53717        +       0
    Intr    53870   54149        +       0
    Intr    54243   54409        +       0
    Term    54518   54823        +       0

>3319339       /27199

   len =     2013   nex =       6

    Init    70541   70591        +       0
    Intr    70680   70806        +       0
    Intr    70898   71067        +       0
    Intr    71347   71626        +       0
    Intr    71727   71875        +       0
    Term    71991   72346        +       0
```

```
>3319339        /19839

   len =     1038   nex =        2

    Term   86289   85954         -         0
    Init   86991   86748         -         0

>3319365        /26983

   len =     1471   nex =        5

    Term   25198   24914         -         0
    Intr   25328   25285         -         0
    Intr   25499   25442         -         0
    Intr   25917   25824         -         0
    Init   26384   26126         -         0

>3319365        /113133

   len =     1059   nex =        1

    Sngl   39553   39787         +         0

>3319365        /37317

   len =      472   nex =        1

    Sngl   39061   38590         -         0

>3319365        /33945

   len =      370   nex =        1

    Sngl   46052   45690         -         0

>3327922        /101361

   len =      938   nex =        2

    Term   25827   25600         -         0
    Init   26537   25924         -         0

>3327922        /38439

   len =     2141   nex =        5

    Init   32746   32973         +         0
    Intr   33067   33167         +         0
    Intr   33246   33289         +         0
    Intr   33713   34348         +         0
    Term   34423   34886         +         0

>3327922        /19349

   len =      591   nex =        1

    Sngl   35078   35668         +         0

>3327922        /27727
```

```
     len =     1390    nex =        5

      Init    39098    39224         +         0
      Intr    39314    39394         +         0
      Intr    39746    39866         +         0
      Intr    39971    40249         +         0
      Term    40408    40487         +         0

 >3327922         /2187

     len =      685    nex =        1

      Sngl    46107    46791         +         0

 >3327922         /38395

     len =     2871    nex =        3

      Init    56836    57097         +         0
      Intr    58412    58924         +         0
      Term    59024    59694         +         0

 >3327922         /3811

     len =     1065    nex =        4

      Init    69508    69568         +         0
      Intr    69677    69792         +         0
      Intr    69891    69954         +         0
      Term    70458    70572         +         0

 >3327922         /12469

     len =     1489    nex =        4

      Init    69508    69568         +         0
      Intr    69677    69792         +         0
      Intr    69891    69954         +         0
      Term    70458    70996         +         0

 >3327922         /18513

     len =     1765    nex =        7

      Init    71210    71356         +         0
      Intr    71451    71651         +         0
      Intr    71857    71917         +         0
      Intr    72008    72209         +         0
      Intr    72320    72441         +         0
      Intr    72534    72638         +         0
      Term    72732    72974         +         0

 >3327922         /14549

     len =      925    nex =        1

      Sngl    80147    81071         +         0

 >3327922         /29298
```

```
  len =     831   nex =       1

   Sngl   80171   81001         +        0

>3327922        /34656

  len =     801   nex =       1

   Sngl   80201   81001         +        0

>3327922        /39583

  len =     490   nex =       1

   Sngl   80576   81064         +        0

>3327922        /13054

  len =     370   nex =       1

   Sngl   80640   80995         +        0

>3335331        /5586

  len =     793   nex =       3

   Init   17573   17712         +        0
   Intr   17853   18139         +        0
   Term   18220   18365         +        0

>3335331        /32284

  len =    4400   nex =      11

   Init    2322    2529         +        0
   Intr    2902    3011         +        0
   Intr    3108    3218         +        0
   Intr    3352    3416         +        0
   Intr    3502    3583         +        0
   Intr    4342    4416         +        0
   Intr    4842    4912         +        0
   Intr    5033    5115         +        0
   Intr    5687    5751         +        0
   Intr    5986    6060         +        0
   Term    6374    6721         +        0

>3335331        /4326

  len =     953   nex =       2

   Term   64184   63680         -        0
   Init   64632   64297         -        0

>3335331        /6906

  len =    3250   nex =       8

   Init    7810    8237         +        0
   Intr    8419    8481         +        0
```

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```
      Intr     8573     8668         +          0
      Intr     9068     9115         +          0
      Intr     9290     9361         +          0
      Intr    10005    10063         +          0
      Intr    10270    10360         +          0
      Term    10857    11050         +          0

>3335331         /19247

    len =      3193    nex =        8

      Init     7832     8237         +          0
      Intr     8419     8481         +          0
      Intr     8573     8668         +          0
      Intr     9068     9115         +          0
      Intr     9290     9361         +          0
      Intr    10005    10063         +          0
      Intr    10270    10360         +          0
      Term    10857    11024         +          0

>3335331         /118658

    len =      1125    nex =        2

      Term    79626    79511         -          0
      Init    80234    80142         -          0

>3335331         /42267

    len =       939    nex =        2

      Init    81876    82398         +          0
      Term    82623    82814         +          0

>3335331         /17230

    len =      1210    nex =        1

      Sngl    83926    85126         +          0

>3335356         /23238

    len =      1810    nex =        2

      Term    99574    98995         -          0
      Init   100803   100401         -          0

>3335356         /31586

    len =      4099    nex =        8

      Init    40725    40991         +          0
      Intr    41490    41675         +          0
      Intr    41764    41876         +          0
      Intr    41973    42156         +          0
      Intr    42435    42532         +          0
      Intr    43054    43261         +          0
      Intr    43348    43605         +          0
      Term    44286    44823         +          0
```

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```
>3335356        /4723

   len =      711    nex =        3

    Init   48610    48831         +         0
    Intr   48899    48963         +         0
    Term   49052    49320         +         0

>3335356        /15751

   len =     4072    nex =       10

    Term   57235    56932         -         0
    Intr   58046    57956         -         0
    Intr   58325    58155         -         0
    Intr   58546    58430         -         0
    Intr   58799    58671         -         0
    Intr   59124    58900         -         0
    Intr   59544    59348         -         0
    Intr   59678    59650         -         0
    Intr   60330    60263         -         0
    Init   61003    60621         -         0

>3335356        /7394

   len =     1030    nex =        2

    Init   65862    66005         +         0
    Term   66091    66596         +         0

>3335356        /25934

   len =      910    nex =        3

    Init   67764    68104         +         0
    Intr   68204    68310         +         0
    Term   68403    68673         +         0

>3335356        /19998

   len =     1039    nex =        1

    Sngl   79183    80221         +         0

>3337347        /4598

   len =     2715    nex =        7

    Init   32576    32711         +         0
    Intr   33094    33465         +         0
    Intr   33623    33850         +         0
    Intr   33937    34291         +         0
    Intr   34378    34502         +         0
    Intr   34599    34734         +         0
    Term   34820    35288         +         0

>3337347        /36037

   len =     1937    nex =        6
```

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```
    Init   33121   33465         +        0
    Intr   33623   33850         +        0
    Intr   33937   34291         +        0
    Intr   34378   34502         +        0
    Intr   34599   34734         +        0
    Term   34820   35057         +        0

>3337347        /37499

  len =     2676   nex =       14

    Init   36337   36480         +        0
    Intr   36582   36614         +        0
    Intr   36700   36764         +        0
    Intr   36846   36933         +        0
    Intr   37031   37103         +        0
    Intr   37379   37485         +        0
    Intr   37592   37691         +        0
    Intr   37841   37917         +        0
    Intr   38020   38068         +        0
    Intr   38160   38206         +        0
    Intr   38297   38367         +        0
    Intr   38476   38531         +        0
    Intr   38603   38732         +        0
    Term   38833   39012         +        0

>3337347        /16411

  len =     2129   nex =        3

    Term   48472   48232         -        0
    Intr   49760   49222         -        0
    Init   50360   50200         -        0

>3337347        /29146

  len =      702   nex =        1

    Sngl   60015   59314         -        0

>3337347        /23727

  len =      979   nex =        1

    Sngl   95243   96221         +        0

>3341671        /105595

  len =     1168   nex =        2

    Term   32434   32064         -        0
    Init   32805   32715         -        0

>3341671        /39499

  len =     2552   nex =        6

    Term   32434   32032         -        0
    Intr   32805   32715         -        0
    Intr   33301   33190         -        0
```

```
        Intr   33563   33475          -          0
        Intr   33731   33661          -          0
        Init   34583   34277          -          0

>3341671        /5217

      len =      610    nex =        2

        Init   39041   39309          +          0
        Term   39394   39647          +          0

>3341671        /30696

      len =      133    nex =        1

        Sngl   41075   40943          -          0

>3341671        /40641

      len =      869    nex =        2

        Init   45689   45897          +          0
        Term   46011   46557          +          0

>3341671        /111157

      len =      610    nex =        2

        Init   45812   45897          +          0
        Term   46011   46420          +          0

>3341671        /40953

      len =     1719    nex =        5

        Term   47540   47148          -          0
        Intr   47874   47709          -          0
        Intr   48054   47945          -          0
        Intr   48358   48140          -          0
        Init   48866   48453          -          0

>3341671        /1136

      len =      314    nex =        1

        Sngl   58089   57776          -          0

>3341671        /42237

      len =     1709    nex =        4

        Term   68153   67782          -          0
        Intr   68844   68674          -          0
        Intr   69172   69084          -          0
        Init   69484   69280          -          0

>3341671        /34828

      len =     1345    nex =        6
```

```
    Init   72023   72154        +        0
    Intr   72231   72327        +        0
    Intr   72444   72516        +        0
    Intr   72596   72697        +        0
    Intr   72783   73032        +        0
    Term   73118   73367        +        0

>3341671        /36996

   len =    3070   nex =        6

    Init   78677   78786        +        0
    Intr   78936   79004        +        0
    Intr   79193   79308        +        0
    Intr   79630   79750        +        0
    Intr   80100   80194        +        0
    Term   80294   80892        +        0

>3341671        /114909

   len =    1518   nex =        6

    Init   78682   78786        +        0
    Intr   78936   79004        +        0
    Intr   79193   79308        +        0
    Intr   79376   79445        +        0
    Intr   79630   79750        +        0
    Term   80100   80194        +        0

>3341671        /19760

   len =    1278   nex =        2

    Term   83127   82506        -        0
    Init   83783   83570        -        0

>3355463        /11583

   len =    1192   nex =        4

    Term   19567   19377        -        0
    Intr   19829   19673        -        0
    Intr   20349   20214        -        0
    Init   20568   20449        -        0

>3355463        /124576

   len =     894   nex =        2

    Init   24459   24491        +        0
    Term   24870   25152        +        0

>3355463        /22479

   len =    1198   nex =        2

    Init   44058   44421        +        0
    Term   44510   44770        +        0

>3355463        /27485
```

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```
    len =    1150    nex =      1

     Sngl   43581   44721        +        0

>3355463       /16403

    len =    1510    nex =      8

     Init   44997   45084        +        0
     Intr   45167   45248        +        0
     Intr   45334   45438        +        0
     Intr   45532   45605        +        0
     Intr   45692   45814        +        0
     Intr   45902   45973        +        0
     Intr   46058   46154        +        0
     Term   46240   46506        +        0

>3355463       /3850

    len =    1187    nex =      5

     Term   60749   60655        -        0
     Intr   61079   60839        -        0
     Intr   61271   61155        -        0
     Intr   61421   61359        -        0
     Init   61841   61597        -        0

>3355463       /15933

    len =    2560    nex =      9

     Term   90887   90794        -        0
     Intr   91034   90970        -        0
     Intr   91211   91128        -        0
     Intr   91605   91540        -        0
     Intr   91892   91758        -        0
     Intr   92227   92182        -        0
     Intr   92490   92384        -        0
     Intr   93059   93011        -        0
     Init   93353   93139        -        0

>3366536       /27918

    len =    1725    nex =      6

     Term   26503   26130        -        0
     Intr   26880   26613        -        0
     Intr   27116   26996        -        0
     Intr   27259   27206        -        0
     Intr   27499   27394        -        0
     Init   27854   27748        -        0

>3366536       /32438

    len =     755    nex =      4

     Init   32527   32606        +        0
     Intr   32715   32774        +        0
     Intr   32882   32935        +        0
```

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```
      Term    33014   33281          +         0

   >3366536        /5485

      len =     1553   nex =         2

      Term    73632   73175          -         0
      Init    74390   73723          -         0

   >3367500        /11257

      len =     1890   nex =         2

      Init    11335   11645          +         0
      Term    11808   12594          +         0

   >3367500        /125642

      len =     2198   nex =         4

      Init    27603   28220          +         0
      Intr    28298   28420          +         0
      Intr    28512   28628          +         0
      Term    29428   29800          +         0

   >3367500        /21771

      len =     2129   nex =         4

      Init    27671   28220          +         0
      Intr    28298   28420          +         0
      Intr    28512   28628          +         0
      Term    29428   29799          +         0

   >3367500        /104934

      len =     1930   nex =         4

      Init    27736   28220          +         0
      Intr    28298   28420          +         0
      Intr    28512   28628          +         0
      Term    29428   29665          +         0

   >3367500        /25284

      len =      865   nex =         3

      Init    27765   28220          +         0
      Intr    28298   28420          +         0
      Term    28512   28629          +         0

   >3367500        /37435

      len =     2016   nex =        10

      Term    29954   29748          -         0
      Intr    30174   30040          -         0
      Intr    30390   30253          -         0
      Intr    30575   30469          -         0
      Intr    30799   30669          -         0
```

```
     Intr   30915   30875         -         0
     Intr   31085   30985         -         0
     Intr   31254   31167         -         0
     Intr   31505   31404         -         0
     Init   31763   31585         -         0

>3367500          /35575

    len =      281    nex =        1

     Sngl   44549   44829         +         0

>3367567          /95636

    len =      411    nex =        1

     Sngl       1     411         +         0

>3367567          /148676

    len =     1341    nex =        4

     Term   16182   16130         -         0
     Intr   16440   16386         -         0
     Intr   16623   16539         -         0
     Init   17470   16947         -         0

>3367567          /16204

    len =     1178    nex =        6

     Term   31280   31024         -         0
     Intr   31447   31367         -         0
     Intr   31568   31524         -         0
     Intr   31726   31669         -         0
     Intr   31913   31827         -         0
     Init   32050   31996         -         0

>3367567          /31322

    len =     1172    nex =        6

     Term   31280   31030         -         0
     Intr   31447   31367         -         0
     Intr   31568   31524         -         0
     Intr   31726   31669         -         0
     Intr   31913   31827         -         0
     Init   32050   31996         -         0

>3367567          /34272

    len =     1514    nex =        1

     Sngl   48006   49519         +         0

>3367567          /4745

    len =      614    nex =        2

     Term   74205   73805         -         0
```

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```
        Init    74418   74292          -         0

      >3367567        /17117

        len =     1216    nex =        3

        Term    84942   84402          -         0
        Intr    85322   85058          -         0
        Init    85617   85409          -         0

      >3367567        /42841

        len =     2110    nex =        7

        Term    86680   86417          -         0
        Intr    86891   86754          -         0
        Intr    87123   86976          -         0
        Intr    87386   87274          -         0
        Intr    87739   87483          -         0
        Intr    88305   88020          -         0
        Init    88518   88382          -         0

      >3367567        /13699

        len =     1019    nex =        4

        Init    88880   89082          +         0
        Intr    89175   89371          +         0
        Intr    89462   89532          +         0
        Term    89633   89686          +         0

      >3386593        /41984

        len =      562    nex =        1

        Sngl    14092   14653          +         0

      >3386593        /82

        len =      140    nex =        1

        Sngl    16606   16467          -         0

      >3386593        /19844

        len =     1150    nex =        3

        Init     4750    5187          +         0
        Intr     5328    5456          +         0
        Term     5544    5890          +         0

      >3386593        /18854

        len =     1713    nex =        4

        Init    62237   62493          +         0
        Intr    63184   63237          +         0
        Intr    63333   63420          +         0
        Term    63493   63944          +         0
```

```
>3386593        /95396

   len =    1213   nex =       3

     Init   62306   62493        +        0
     Intr   63184   63237        +        0
     Term   63333   63420        +        0

>3386593        /998

   len =    2124   nex =      10

     Term   69215   69011        -        0
     Intr   69358   69307        -        0
     Intr   69546   69457        -        0
     Intr   69727   69632        -        0
     Intr   69907   69808        -        0
     Intr   70047   69992        -        0
     Intr   70209   70174        -        0
     Intr   70369   70293        -        0
     Intr   70834   70768        -        0
     Init   71134   70927        -        0

>3386593        /13925

   len =    1676   nex =       3

     Init    7808    7937        +        0
     Intr    8704    9013        +        0
     Term    9133    9483        +        0

>3386593        /12250

   len =    1667   nex =       7

     Term   85697   85517        -        0
     Intr   86125   85952        -        0
     Intr   86285   86211        -        0
     Intr   86455   86375        -        0
     Intr   86624   86550        -        0
     Intr   86781   86707        -        0
     Init   87183   86857        -        0

>3386593        /12487

   len =    1630   nex =       6

     Term   85849   85656        -        0
     Intr   86125   85952        -        0
     Intr   86285   86211        -        0
     Intr   86455   86375        -        0
     Intr   86624   86550        -        0
     Init   86781   86707        -        0

>3386593        /9000

   len =    2770   nex =      13

     Init   87778   87938        +        0
     Intr   88044   88171        +        0
```

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```
     Intr   88293   88398        +         0
     Intr   88487   88568        +         0
     Intr   88661   88731        +         0
     Intr   88818   88859        +         0
     Intr   88982   89106        +         0
     Intr   89196   89289        +         0
     Intr   89407   89482        +         0
     Intr   89599   89762        +         0
     Intr   89840   89945        +         0
     Intr   90121   90207        +         0
     Term   90311   90531        +         0

>3395421       /123915

    len =     625   nex =       2

     Init   14364   14582        +         0
     Term   14746   14988        +         0

>3395421       /14329

    len =     879   nex =       3

     Term   18618   18349        -         0
     Intr   18781   18712        -         0
     Init   19227   18940        -         0

>3395421       /43031

    len =    1786   nex =       4

     Term   40343   39798        -         0
     Intr   40877   40709        -         0
     Intr   41167   40976        -         0
     Init   41583   41311        -         0

>3395421       /25615

    len =     833   nex =       1

     Sngl   50675   51507        +         0

>3395421       /16674

    len =    1056   nex =       3

     Init    9467    9820        +         0
     Intr    9908   10031        +         0
     Term   10113   10522        +         0

>3399678       /3807

    len =    2443   nex =       6

     Term    9355    8974        -         0
     Intr    9578    9453        -         0
     Intr    9939    9691        -         0
     Intr   10435   10084        -         0
     Intr   10680   10541        -         0
     Init   11416   10972        -         0
```

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```
>3399678        /39874

   len =     2953    nex =       11

     Init   11842    11976        +         0
     Intr   12277    12410        +         0
     Intr   12562    12643        +         0
     Intr   12772    12839        +         0
     Intr   12966    13067        +         0
     Intr   13154    13243        +         0
     Intr   13590    13664        +         0
     Intr   13766    13798        +         0
     Intr   13901    13978        +         0
     Intr   14081    14137        +         0
     Term   14395    14794        +         0

>3399678        /3234

   len =      315    nex =        1

     Sngl    3776     4090        +         0

>3399678        /12077

   len =     1815    nex =        5

     Init    4154     4420        +         0
     Intr    4549     4621        +         0
     Intr    5205     5269        +         0
     Intr    5617     5726        +         0
     Term    5816     5968        +         0

>3399678        /6672

   len =     3673    nex =       12

     Init   53802    54056        +         0
     Intr   54661    54805        +         0
     Intr   54887    54982        +         0
     Intr   55372    55448        +         0
     Intr   55709    55788        +         0
     Intr   55934    55994        +         0
     Intr   56137    56204        +         0
     Intr   56296    56377        +         0
     Intr   56467    56556        +         0
     Intr   56645    56722        +         0
     Intr   56864    56983        +         0
     Term   57108    57474        +         0

>3399678        /38167

   len =     2082    nex =        9

     Init   55396    55448        +         0
     Intr   55709    55788        +         0
     Intr   55934    55994        +         0
     Intr   56137    56204        +         0
     Intr   56296    56377        +         0
     Intr   56467    56556        +         0
```

```
    Intr    56645    56722        +        0
    Intr    56864    56983        +        0
    Term    57108    57474        +        0

>3399678        /108783

   len =      111    nex =        1

    Sngl     6456     6566        +        0

>3399678        /155960

   len =      513    nex =        1

    Sngl    73631    74143        +        0

>3399678        /13937

   len =     1427    nex =        4

    Init    84754    84947        +        0
    Intr    85039    85251        +        0
    Intr    85353    85508        +        0
    Term    85609    86180        +        0

>3402671        /853

   len =     3156    nex =        9

    Term   104995   104737        -        0
    Intr   105259   105204        -        0
    Intr   105621   105565        -        0
    Intr   106216   106066        -        0
    Intr   106605   106557        -        0
    Intr   106819   106726        -        0
    Intr   106986   106914        -        0
    Intr   107186   107086        -        0
    Init   107892   107260        -        0

>3402671        /39069

   len =     2213    nex =        4

    Init    16497    16715        +        0
    Intr    16919    17386        +        0
    Intr    17497    17952        +        0
    Term    18047    18451        +        0

>3402671        /33545

   len =     2633    nex =        4

    Init    21758    21965        +        0
    Intr    22289    22750        +        0
    Intr    23024    23482        +        0
    Term    23572    23906        +        0

>3402671        /29616

   len =     1257    nex =        6
```

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```
     Term   29445   29258        -        0
     Intr   29604   29548        -        0
     Intr   29812   29699        -        0
     Intr   29980   29930        -        0
     Intr   30161   30064        -        0
     Init   30514   30442        -        0

>3402695       /7469

    len =     1510   nex =       2

     Term   13030   12578        -        0
     Init   13512   13440        -        0

>3402695       /152917

    len =      653   nex =       0

>3402695       /16715

    len =      822   nex =       2

     Term   44889   44516        -        0
     Init   45337   45254        -        0

>3402695       /123659

    len =      945   nex =       3

     Term   49228   48980        -        0
     Intr   49706   49346        -        0
     Init   49924   49810        -        0

>3402695       /35600

    len =     3715   nex =      12

     Term   64962   64623        -        0
     Intr   65129   65078        -        0
     Intr   65432   65316        -        0
     Intr   65870   65778        -        0
     Intr   66348   66129        -        0
     Intr   66853   66694        -        0
     Intr   67168   67094        -        0
     Intr   67504   67317        -        0
     Intr   67667   67586        -        0
     Intr   67847   67766        -        0
     Intr   68048   68014        -        0
     Init   68337   68152        -        0

>3402695       /6727

    len =      897   nex =       1

     Sngl   70904   71800        +        0

>3402745       /33333

    len =     1510   nex =       3
```

```
      Term    24071    23112          -          0
      Intr    24287    24158          -          0
      Init    24617    24377          -          0

>3402745        /21213

    len =      922    nex =         2

      Init    29567    29934          +          0
      Term    30280    30488          +          0

>3402745        /33975

    len =     2309    nex =         7

      Init    30883    31350          +          0
      Intr    31838    31908          +          0
      Intr    31988    32069          +          0
      Intr    32169    32287          +          0
      Intr    32549    32626          +          0
      Intr    32714    32833          +          0
      Term    32923    33191          +          0

>3402745        /10221

    len =     2063    nex =         4

      Init    33374    33615          +          0
      Intr    33835    34236          +          0
      Intr    34556    34970          +          0
      Term    35046    35436          +          0

>3402745        /20924

    len =     2230    nex =         4

      Init    33384    33615          +          0
      Intr    33835    34236          +          0
      Intr    34556    34970          +          0
      Term    35046    35608          +          0

>3402745        /36892

    len =     1308    nex =         3

      Term    51257    50714          -          0
      Intr    51406    51332          -          0
      Init    52021    51488          -          0

>3402745        /36836

    len =     2969    nex =        14

      Term    52493    52292          -          0
      Intr    52706    52584          -          0
      Intr    52859    52793          -          0
      Intr    53091    53007          -          0
      Intr    53395    53343          -          0
      Intr    53658    53611          -          0
```

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```
      Intr    53884    53742         -         0
      Intr    54086    53980         -         0
      Intr    54266    54184         -         0
      Intr    54433    54360         -         0
      Intr    54609    54529         -         0
      Intr    54759    54707         -         0
      Intr    54938    54876         -         0
      Init    55260    55060         -         0

>3402745         /42465

     len =     1648    nex =        1

      Sngl    75185    74973         -         0

>3402745         /35503

     len =     1638    nex =        1

      Sngl    75185    74973         -         0

>3402745         /31790

     len =     2014    nex =        4

      Term    76187    75789         -         0
      Intr    76568    76278         -         0
      Intr    76777    76655         -         0
      Init    77133    76861         -         0

>3402745         /4621

     len =     2027    nex =        5

      Init    80600    80931         +         0
      Intr    81288    81467         +         0
      Intr    81659    81740         +         0
      Intr    81819    82085         +         0
      Term    82176    82626         +         0

>3402745         /95385

     len =      588    nex =        2

      Init    82005    82085         +         0
      Term    82176    82592         +         0

>3402745         /17760

     len =     2350    nex =        9

      Init    85225    85495         +         0
      Intr    85763    85943         +         0
      Intr    86039    86134         +         0
      Intr    86229    86284         +         0
      Intr    86453    86536         +         0
      Intr    86690    86782         +         0
      Intr    86876    86943         +         0
      Intr    87122    87195         +         0
      Term    87314    87572         +         0
```

```
>3402745       /40247

   len =    2298   nex =       9

    Init   90176   90278        +        0
    Intr   90538   90721        +        0
    Intr   90829   90939        +        0
    Intr   91060   91115        +        0
    Intr   91287   91370        +        0
    Intr   91504   91543        +        0
    Intr   91646   91713        +        0
    Intr   91818   91891        +        0
    Term   91975   92274        +        0

>3402747       /5691

   len =    1330   nex =       3

    Init    2703    3023        +        0
    Intr    3396    3691        +        0
    Term    3790    4023        +        0

>3402747       /31930

   len =     564   nex =       2

    Init    3464    3691        +        0
    Term    3790    4016        +        0

>3406034       /37757

   len =    2240   nex =      10

    Term    2322    2126        -        0
    Intr    2505    2408        -        0
    Intr    2729    2583        -        0
    Intr    2889    2828        -        0
    Intr    3253    3163        -        0
    Intr    3389    3336        -        0
    Intr    3580    3494        -        0
    Intr    3801    3673        -        0
    Intr    4116    4046        -        0
    Init    4365    4221        -        0

>3406034       /28528

   len =     775   nex =       1

    Sngl   44408   43634        -        0

>3406034       /102813

   len =    1630   nex =       3

    Init     982    1230        +        0
    Intr    1313    1442        +        0
    Term    1995    2193        +        0

>3406034       /35331
```

```
len =     1690   nex =       4

 Init      565     898          +        0
 Intr      982    1230          +        0
 Intr     1313    1442          +        0
 Term     1995    2253          +        0

>3406034        /2499

len =     1274   nex =       4

 Init    66373   66517          +        0
 Intr    66709   67020          +        0
 Intr    67284   67348          +        0
 Term    67442   67646          +        0

>3406034        /21669

len =     1075   nex =       4

 Term    87265   87035          -        0
 Intr    87552   87477          -        0
 Intr    87871   87810          -        0
 Init    88109   87956          -        0

>3413696        /2474

len =      376   nex =       1

 Sngl    72418   72793          +        0

>3413696        /110411

len =      800   nex =       1

 Sngl    73023   73822          +        0

>3413696        /39666

len =     2010   nex =       9

 Term    74013   73835          -        0
 Intr    74154   74093          -        0
 Intr    74343   74235          -        0
 Intr    74570   74521          -        0
 Intr    74714   74657          -        0
 Intr    74902   74825          -        0
 Intr    75138   75089          -        0
 Intr    75388   75308          -        0
 Init    75844   75677          -        0

>3420042        /31044

len =     1153   nex =       1

 Sngl    16199   15047          -        0

>3420042        /33789
```

```
     len =     1543    nex =        4

      Term   49244    48780          -          0
      Intr   49472    49347          -          0
      Intr   49668    49615          -          0
      Init   50322    49851          -          0

   >3420042          /33195

     len =     1210    nex =        3

      Init   51312    51412          +          0
      Intr   51803    51916          +          0
      Term   52019    52117          +          0

   >3420042          /34564

     len =     1630    nex =        4

      Init   51312    51412          +          0
      Intr   51803    51916          +          0
      Intr   52019    52155          +          0
      Term   52279    52540          +          0

   >3420042          /6397

     len =      913    nex =        1

      Sngl   58119    57207          -          0

   >3420043          /38891

     len =     3670    nex =        5

      Term   32310    31803          -          0
      Intr   33332    33174          -          0
      Intr   33637    33456          -          0
      Intr   34403    34232          -          0
      Init   35464    35021          -          0

   >3420043          /35872

     len =     1066    nex =        0

   >3420043          /22599

     len =     1104    nex =        0

   >3420043          /124634

     len =     2510    nex =        1

      Sngl   41769    41941          +          0

   >3420043          /36979

     len =     3413    nex =        8

      Init   56129    56549          +          0
      Intr   57173    57262          +          0
     Intr    57809    57875         +          0
     Intr    58355    58447         +          0
     Intr    58535    58720         +          0
     Intr    58809    58889         +          0
     Intr    58968    59105         +          0
     Term    59204    59541         +          0

>3420043        /9257

     len =      593    nex =        1

     Sngl    61934    61342         -          0

>3420043        /19211

     len =     1522    nex =        6

     Init    70609    70725         +          0
     Intr    70916    70985         +          0
     Intr    71126    71173         +          0
     Intr    71279    71302         +          0
     Intr    71525    71707         +          0
     Term    71791    72130         +          0

>3420043        /206307

     len =     1090    nex =        5

     Init    70617    70725         +          0
     Intr    70916    70985         +          0
     Intr    71126    71173         +          0
     Intr    71279    71302         +          0
     Term    71525    71706         +          0

>3420043        /150229

     len =     1511    nex =        6

     Init    70617    70725         +          0
     Intr    70916    70985         +          0
     Intr    71126    71173         +          0
     Intr    71279    71302         +          0
     Intr    71525    71707         +          0
     Term    71791    72127         +          0

>3420043        /13487

     len =     1518    nex =        0

>3426033        /10624

     len =     2499    nex =       11

     Init    10293    10440         +          0
     Intr    10665    10760         +          0
     Intr    10841    10909         +          0
     Intr    11001    11105         +          0
     Intr    11183    11239         +          0
     Intr    11323    11523         +          0
     Intr    11684    11776         +          0
```

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```
        Intr    11858    11957          +         0
        Intr    12082    12181          +         0
        Intr    12426    12511          +         0
        Term    12597    12791          +         0

    >3426033        /33835

       len =      393    nex =        2

        Init    29182    29215          +         0
        Term    29293    29574          +         0

    >3426033        /37620

       len =     3550    nex =       11

        Term    57690    57420          -         0
        Intr    57870    57778          -         0
        Intr    58073    58007          -         0
        Intr    58268    58163          -         0
        Intr    58579    58495          -         0
        Intr    59100    59009          -         0
        Intr    59365    59243          -         0
        Intr    59760    59655          -         0
        Intr    60149    60043          -         0
        Intr    60338    60247          -         0
        Init    60965    60797          -         0

    >3445196        /33511

       len =     1643    nex =        4

        Init    35160    35638          +         0
        Intr    35732    35801          +         0
        Intr    36165    36327          +         0
        Term    36415    36802          +         0

    >3445196        /121762

       len =      854    nex =        0

    >3445196        /39568

       len =     3654    nex =       12

        Init    70556    70702          +         0
        Intr    70777    70922          +         0
        Intr    71023    71099          +         0
        Intr    71180    71302          +         0
        Intr    71707    71809          +         0
        Intr    71878    71915          +         0
        Intr    72121    72219          +         0
        Intr    72321    72407          +         0
        Intr    72500    72568          +         0
        Intr    72754    72825          +         0
        Intr    72906    73027          +         0
        Term    73847    74209          +         0

    >3445196        /35
```

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```
    len =     2265    nex =        6

      Init   72121   72219          +         0
      Intr   72321   72407          +         0
      Intr   72500   72568          +         0
      Intr   72754   72825          +         0
      Intr   72906   73027          +         0
      Term   73847   74140          +         0

>3445196        /3181

    len =      558    nex =        2

      Init   82071   82214          +         0
      Term   82390   82431          +         0

>3449311        /5361

    len =     1236    nex =        2

      Term   27604   27255          -         0
      Init   28490   28213          -         0

>3449311        /141753

    len =     1072    nex =        4

      Term   47195   46888          -         0
      Intr   47404   47285          -         0
      Intr   47631   47489          -         0
      Init   47959   47723          -         0

>3449311        /30287

    len =     1117    nex =        4

      Term   47195   46889          -         0
      Intr   47404   47285          -         0
      Intr   47631   47489          -         0
      Init   48005   47723          -         0

>3449311        /121388

    len =     2530    nex =        5

      Init   51403   51752          +         0
      Intr   51838   51965          +         0
      Intr   52072   52305          +         0
      Intr   53291   53537          +         0
      Term   53646   53926          +         0

>3449311        /21192

    len =     2530    nex =        5

      Init   51403   51752          +         0
      Intr   51838   51965          +         0
      Intr   52072   52305          +         0
      Intr   53291   53537          +         0
      Term   53646   53931          +         0
```

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```
>3449312        /114786

   len =    1491   nex =       6

    Init   20409   20522         +        0
    Intr   20834   20899         +        0
    Intr   21007   21151         +        0
    Intr   21246   21464         +        0
    Intr   21559   21772         +        0
    Term   21862   21890         +        0

>3449312        /20725

   len =    2155   nex =       7

    Init   35173   35268         +        0
    Intr   35340   35570         +        0
    Intr   35843   35940         +        0
    Intr   36031   36217         +        0
    Intr   36303   36433         +        0
    Intr   36515   36751         +        0
    Term   36841   37327         +        0

>3449312        /2159

   len =     881   nex =       1

    Sngl   40080   40960         +        0

>3449312        /11266

   len =    2506   nex =       4

    Init   70384   70926         +        0
    Intr   71299   71633         +        0
    Intr   71731   71930         +        0
    Term   72582   72889         +        0

>3449312        /37540

   len =    2540   nex =       8

    Init    8228    8379         +        0
    Intr    8897    8980         +        0
    Intr    9081    9301         +        0
    Intr    9387    9465         +        0
    Intr    9654    9823         +        0
    Intr    9965   10151         +        0
    Intr   10243   10320         +        0
    Term   10403   10767         +        0

>3449313        /40718

   len =     743   nex =       2

    Term   17412   16864         -        0
    Init   17606   17499         -        0

>3449313        /6699
```

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```
     len =     2291   nex =        3

      Term   19583   18826         -         0
      Intr   20255   19887         -         0
      Init   21116   20331         -         0

 >3449313        /31573

     len =     1964   nex =        3

      Init   27831   28026         +         0
      Intr   28183   28618         +         0
      Term   29403   29794         +         0

 >3449313        /147765

     len =      831   nex =        1

      Sngl   55558   54728         -         0

 >3449314        /25475

     len =      698   nex =        1

      Sngl    1344     647         -         0

 >3449314        /6982

     len =     1359   nex =        2

      Term    6894    6520         -         0
      Init    7878    7794         -         0

 >3449315        /29551

     len =     1560   nex =        5

      Term    2958    2620         -         0
      Intr    3194    3059         -         0
      Intr    3363    3280         -         0
      Intr    3509    3452         -         0
      Init    4179    3608         -         0

 >3449316        /107101

     len =      391   nex =        1

      Sngl   46435   46825         +         0

 >3449316        /17050

     len =      477   nex =        1

      Sngl    8487    8751         +         0

 >3449317        /3729

     len =     2698   nex =        9
     Term   36464   36043          -         0
     Intr   36675   36563          -         0
     Intr   36858   36770          -         0
     Intr   37059   36954          -         0
     Intr   37343   37287          -         0
     Intr   37908   37819          -         0
     Intr   38239   38014          -         0
     Intr   38401   38368          -         0
     Init   38713   38596          -         0

>3449317        /2842

   len =    1664   nex =        3

     Init   38942   39208          +         0
     Intr   39996   40060          +         0
     Term   40346   40605          +         0

>3449317        /91885

   len =     643   nex =        3

     Init    5652    5704          +         0
     Intr    5785    5868          +         0
     Term    5959    6289          +         0

>3449317        /21756

   len =    2140   nex =        7

     Init    6801    6845          +         0
     Intr    7257    7326          +         0
     Intr    7438    7656          +         0
     Intr    7745    7824          +         0
     Intr    7917    8033          +         0
     Intr    8150    8454          +         0
     Term    8552    8940          +         0

>3449318        /1523

   len =     931   nex =        3

     Term   21868   21538          -         0
     Intr   22060   21983          -         0
     Init   22468   22163          -         0

>3449320        /31551

   len =     954   nex =        3

     Init   22442   22860          +         0
     Intr   23003   23177          +         0
     Term   23304   23395          +         0

>3449320        /14044

   len =    1603   nex =        4

     Init   37144   37440          +         0
     Intr   38083   38151          +         0
```

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```
     Intr    38254   38360        +        0
     Term    38471   38746        +        0

>3449320        /27973

    len =     1526   nex =        4

     Init    48903   49084        +        0
     Intr    49585   49623        +        0
     Intr    49797   50027        +        0
     Term    50116   50428        +        0

>3449320        /12322

    len =      524   nex =        1

     Sngl    60298   59775        -        0

>3449320        /4875

    len =      984   nex =        2

     Init    62405   62653        +        0
     Term    63001   63388        +        0

>3449321        /32212

    len =      638   nex =        1

     Sngl    19476   20102        +        0

>3449321        /6441

    len =     2658   nex =        2

     Term    33609   32653        -        0
     Init    35310   34793        -        0

>3449321        /27795

    len =      850   nex =        2

     Term    36545   36330        -        0
     Init    37179   36861        -        0

>3449321        /14414

    len =     1495   nex =        4

     Init    43130   43391        +        0
     Intr    43495   43751        +        0
     Intr    43847   43993        +        0
     Term    44204   44624        +        0

>3449322        /149970

    len =     1056   nex =        2

     Term    15835   15245        -        0
     Init    16155   15991        -        0
```

```
>3449322        /970

   len =      496    nex =        1

    Sngl      994      499        -         0

>3449323        /35890

   len =     1873    nex =        3

    Init   27590    27970         +         0
    Intr   28290    28562         +         0
    Term   28595    28757         +         0
>3449323        /17159

   len =     1630    nex =        2

    Init   32944    33507         +         0
    Term   34103    34565         +         0

>3449323        /21382

   len =      276    nex =        1

    Sngl   34337    34612         +         0

>3449323        /42747

   len =     2074    nex =        4

    Term   35032    34594         -         0
    Intr   35437    35219         -         0
    Intr   35698    35519         -         0
    Init   36667    36397         -         0

>3449323        /39065

   len =      869    nex =        0
>3449323        /13801

   len =     2151    nex =        7

    Term   52032    51755         -         0
    Intr   52225    52124         -         0
    Intr   52436    52311         -         0
    Intr   52789    52529         -         0
    Intr   53298    52875         -         0
    Intr   53623    53505         -         0
    Init   53905    53724         -         0

>3449323        /122618

   len =     1210    nex =        3

    Term   69206    68963         -         0
    Intr   69709    69545         -         0
    Init   69969    69811         -         0
```

```
>3449323        /13730

   len =     1956    nex =        2

     Term   71264   70606          -        0
     Init   71787   71394          -        0

>3449324        /19092

   len =      710    nex =        3

     Init   22002   22115          +        0
     Intr   22241   22398          +        0
     Term   22512   22711          +        0

>3449325        /21890

   len =     1523    nex =        3

     Term   34360   33685          -        0
     Intr   34566   34431          -        0
     Init   35207   34772          -        0

>3449325        /42965

   len =     1538    nex =        3

     Term   34360   33734          -        0
     Intr   34566   34431          -        0
     Init   35271   34772          -        0

>3449325        /13806

   len =     1256    nex =        3

     Term   40783   40075          -        0
     Intr   41008   40873          -        0
     Init   41330   41098          -        0

>3449326        /119717

   len =      656    nex =        2

     Term   23133   22788          -        0
     Init   23443   23373          -        0

>3449326        /3086

   len =     1090    nex =        2

     Init   46084   46198          +        0
     Term   46486   47171          +        0

>3449326        /42926

   len =     2685    nex =        9

     Term   61606   61430          -        0
     Intr   61803   61717          -        0
```

```
      Intr   61962   61888        -         0
      Intr   62569   62465        -         0
      Intr   62770   62672        -         0
      Intr   62992   62859        -         0
      Intr   63168   63090        -         0
      Intr   63494   63276        -         0
      Init   64114   63864        -         0

>3449326       /120947

     len =    1295   nex =       2

      Init   71680   72166        +         0
      Term   72722   72974        +         0

>3449327       /26543

     len =     395   nex =       1

      Sngl   14331   13937        -         0

>3449327       /37055

     len =    2110   nex =      10

      Term   20457   20120        -         0
      Intr   20670   20567        -         0
      Intr   20855   20756        -         0
      Intr   21036   20939        -         0
      Intr   21174   21122        -         0
      Intr   21586   21515        -         0
      Intr   21744   21673        -         0
      Intr   21920   21825        -         0
      Intr   22057   21990        -         0
      Init   22225   22134        -         0

>3449327       /27492

     len =    1820   nex =       4

      Init   26052   26288        +         0
      Intr   26367   26724        +         0
      Intr   26809   27384        +         0
      Term   27475   27871        +         0

>3449327       /11250

     len =    1693   nex =       1

      Sngl   35338   34719        -         0

>3449327       /157644

     len =    1090   nex =       3

      Term    5305    5089        -         0
      Intr    5612    5397        -         0
      Init    5960    5886        -         0

>3449327       /14401
```

```
     len =     1180    nex =        3

      Term    5305     5037          -          0
      Intr    5612     5397          -          0
      Init    5960     5886          -          0

>3449327         /38345

     len =     1496    nex =        3

      Term   61922    61607          -          0
      Intr   62622    62493          -          0
      Init   63102    62707          -          0

>3449327         /12989

     len =     1634    nex =        1

      Sngl   65098    64162          -          0

>3449327         /39633

     len =     1858    nex =        3

      Init   65970    66124          +          0
      Intr   66222    66328          +          0
      Term   67542    67827          +          0

>3449327         /3226

     len =     1630    nex =        3

      Init    7695     7813          +          0
      Intr    7943     8038          +          0
      Term    8118     8562          +          0

>3449327         /14416

     len =     1425    nex =        3

      Init    7695     7813          +          0
      Intr    7943     8038          +          0
      Term    8118     8560          +          0

>3449329         /99348

     len =      771    nex =        1

      Sngl   22128    21358          -          0

>3449329         /39355

     len =      456    nex =        1

      Sngl   22146    21709          -          0

>3449329         /34273

     len =     1101    nex =        1
```

```
    Sngl    3712    2612         -         0

>3449329        /33707

   len =     1699   nex =        5

    Init   47513   47739         +         0
    Intr   47826   48010         +         0
    Intr   48122   48322         +         0
    Intr   48410   48571         +         0
    Term   48642   49211         +         0

>3449329        /117597

   len =     1750   nex =        1

    Sngl   47513   47739         +         0

>3449329        /112975

   len =      656   nex =        1

    Sngl   77611   76956         -         0

>3449329        /7878

   len =     2318   nex =        3

    Init   79072   79208         +         0
    Intr   79974   80125         +         0
    Term   80277   81389         +         0

>3449329        /110801

   len =      575   nex =        2

    Term   83763   83571         -         0
    Init   84145   84015         -         0

>3449330        /11513

   len =     1658   nex =        3

    Term   63037   62811         -         0
    Intr   63717   63115         -         0
    Init   64468   64216         -         0

>3449331        /18210

   len =     1246   nex =        3

    Term   14046   13722         -         0
    Intr   14527   14422         -         0
    Init   14967   14798         -         0

>3449331        /35596

   len =     1469   nex =        2
```

```
    Init   16127   16294        +       0
    Term   17028   17595        +       0

>3449331       /6591

   len =    2293   nex =       2

    Init   18665   19043        +       0
    Term   19547   20106        +       0

>3449331       /31901

   len =    2110   nex =      11

    Term   55268   55105        -       0
    Intr   55402   55362        -       0
    Intr   55630   55500        -       0
    Intr   55809   55716        -       0
    Intr   55980   55903        -       0
    Intr   56162   56074        -       0
    Intr   56315   56251        -       0
    Intr   56488   56397        -       0
    Intr   56676   56589        -       0
    Intr   56832   56746        -       0
    Init   57213   57145        -       0

>3449331       /7901

   len =    2453   nex =      11

    Term   55268   54818        -       0
    Intr   55402   55362        -       0
    Intr   55630   55500        -       0
    Intr   55809   55716        -       0
    Intr   55980   55903        -       0
    Intr   56162   56074        -       0
    Intr   56315   56251        -       0
    Intr   56488   56397        -       0
    Intr   56676   56589        -       0
    Intr   56832   56746        -       0
    Init   57270   57145        -       0

>3449331       /6701

   len =    2621   nex =      11

    Term   55268   54844        -       0
    Intr   55402   55362        -       0
    Intr   55630   55500        -       0
    Intr   55809   55716        -       0
    Intr   55980   55903        -       0
    Intr   56162   56074        -       0
    Intr   56315   56251        -       0
    Intr   56488   56397        -       0
    Intr   56676   56589        -       0
    Intr   56832   56746        -       0
    Init   57464   57342        -       0

>3449331       /24060
```

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```
      len =    1669   nex =       5

        Term   59217   58869        -         0
        Intr   59381   59304        -         0
        Intr   59645   59545        -         0
        Intr   59840   59743        -         0
        Init   60337   60235        -         0

   >3449331       /19444

      len =     701   nex =       2

        Init   62474   62588        +         0
        Term   62729   62796        +         0

   >3449331       /43057

      len =     653   nex =       2

        Term   64577   64471        -         0
        Init   65123   64716        -         0

   >3449331       /7828

      len =     713   nex =       2

        Init   68243   68660        +         0
        Term   68755   68955        +         0

   >3449331       /16326

      len =     702   nex =       2

        Init   68246   68660        +         0
        Term   68755   68947        +         0

   >3449331       /28773

      len =     699   nex =       2

        Init   68251   68660        +         0
        Term   68755   68949        +         0

   >3449331       /94898

      len =     310   nex =       1

        Sngl   68272   68572        +         0

   >3449332       /31894

      len =    1677   nex =       0

   >3449332       /37326

      len =    2911   nex =      13

        Term   30818   30775        -         0
        Intr   31080   30961        -         0
        Intr   31445   31341        -         0
```

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```
     Intr   31611   31534        -       0
     Intr   31753   31688        -       0
     Intr   31902   31828        -       0
     Intr   32046   31984        -       0
     Intr   32231   32142        -       0
     Intr   32404   32308        -       0
     Intr   32674   32577        -       0
     Intr   32859   32748        -       0
     Intr   32971   32946        -       0
     Init   33267   33058        -       0

>3449333       /37806

    len =     1510    nex =       1

     Sngl   32409   33912        +       0

>3449333       /39990

    len =     1218    nex =       1

     Sngl   35415   34198        -       0

>3449334       /37422

    len =     1129    nex =       3

     Term   10778   10442        -       0
     Intr   10889   10867        -       0
     Init   11570   11474        -       0

>3449334       /11092

    len =      432    nex =       1

     Sngl   38534   38322        -       0

>3449334       /21208

    len =     1390    nex =       6

     Init   48947   49088        +       0
     Intr   49267   49375        +       0
     Intr   49516   49593        +       0
     Intr   49742   49775        +       0
     Intr   49871   49939        +       0
     Term   50057   50336        +       0

>3449334       /12613

    len =     1460    nex =       3

     Init   59084   59287        +       0
     Intr   59884   60153        +       0
     Term   60375   60543        +       0

>3449334       /9267

    len =      550    nex =       2
    Init    6629    6820         +        0
    Term    6919    7170         +        0

>3449334        /6477

   len =     1114    nex =       2

    Init   75520   75670         +        0
    Term   76364   76633         +        0

>3449334        /22052

   len =      503    nex =       2

    Init   77334   77521         +        0
    Term   77615   77836         +        0

>3449334        /154063

   len =     1339    nex =       5

    Term   78133   77804         -        0
    Intr   78297   78209         -        0
    Intr   78506   78373         -        0
    Intr   78651   78601         -        0
    Init   79142   78752         -        0

>3449334        /4763

   len =     1158    nex =       2

    Init   80747   81302         +        0
    Term   81744   81782         +        0

>3451055        /3982

   len =     1589    nex =       4

    Init   32795   33172         +        0
    Intr   33446   33741         +        0
    Intr   33836   33976         +        0
    Term   34110   34383         +        0

>3451055        /4578

   len =     2544    nex =       8

    Init   41121   41326         +        0
    Intr   42072   42303         +        0
    Intr   42380   42459         +        0
    Intr   42577   42730         +        0
    Intr   42805   42883         +        0
    Intr   42968   43055         +        0
    Intr   43172   43218         +        0
    Term   43361   43664         +        0

>3451055        /32181

   len =     2451    nex =       8
```

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```
    Init   41200   41326        +        0
    Intr   42072   42303        +        0
    Intr   42380   42459        +        0
    Intr   42607   42730        +        0
    Intr   42805   42883        +        0
    Intr   42968   43055        +        0
    Intr   43172   43218        +        0
    Term   43361   43650        +        0

>3451055        /25694

  len =    2230   nex =       8

    Init   61121   61435        +        0
    Intr   61520   61541        +        0
    Intr   61868   61924        +        0
    Intr   62011   62092        +        0
    Intr   62316   62422        +        0
    Intr   62528   62652        +        0
    Intr   62751   62769        +        0
    Term   62860   63348        +        0

>3451055        /104784

  len =     730   nex =       2

    Term   70038   69729        -        0
    Init   70454   70123        -        0

>3451055        /9836

  len =     506   nex =       1

    Sngl   78727   79232        +        0

>3461810        /11805

  len =    2369   nex =       8

    Term   42733   42323        -        0
    Intr   42938   42849        -        0
    Intr   43131   43045        -        0
    Intr   43376   43226        -        0
    Intr   43531   43464        -        0
    Intr   43911   43840        -        0
    Intr   44222   44021        -        0
    Init   44691   44486        -        0

>3461810        /27242

  len =    1289   nex =       4

    Init   53744   54065        +        0
    Intr   54142   54297        +        0
    Intr   54403   54601        +        0
    Term   54682   55032        +        0

>3461810        /10750

  len =    1243   nex =       2
```

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```
    Term    7067    6782        -        0
    Init    8024    7769        -        0

>3461810        /2881

   len =     1758   nex =        3

    Term   97905   97123        -        0
    Intr   98453   98141        -        0
    Init   98676   98543        -        0

>3461834        /17914

   len =     1906   nex =        4

    Term   11675   11426        -        0
    Intr   11885   11695        -        0
    Intr   12878   12560        -        0
    Init   13331   13074        -        0

>3461834        /38091

   len =      472   nex =        1

    Sngl   29436   28965        -        0

>3461834        /10164

   len =     1913   nex =        4

    Init   49465   49733        +        0
    Intr   50347   50450        +        0
    Intr   50887   51016        +        0
    Term   51126   51377        +        0

>3461834        /30975

   len =     2230   nex =        6

    Init   57851   58312        +        0
    Intr   58588   58751        +        0
    Intr   58845   59008        +        0
    Intr   59093   59352        +        0
    Intr   59440   59566        +        0
    Term   59735   60074        +        0

>3482964        /39928

   len =     2424   nex =        7

    Term   23256   22843        -        0
    Intr   23516   23396        -        0
    Intr   23815   23590        -        0
    Intr   23978   23891        -        0
    Intr   24191   24068        -        0
    Intr   24802   24731        -        0
    Init   24971   24885        -        0

>3482964        /40987
```

```
    len =    1881   nex =       6

     Init  27474   27850        +        0
     Intr  27982   28122        +        0
     Intr  28285   28425        +        0
     Intr  28664   28713        +        0
     Intr  28805   28934        +        0
     Term  29067   29354        +        0

>3482964        /31673

    len =     263   nex =       1

     Sngl    5556   5818        +        0

>3482964        /14105

    len =    1707   nex =       3

     Init  77290   77640        +        0
     Intr  78070   78357        +        0
     Term  78446   78996        +        0

>3482964        /3709

    len =    1403   nex =       6

     Term   7020    6611        -        0
     Intr   7158    7104        -        0
     Intr   7297    7245        -        0
     Intr   7457    7381        -        0
     Intr   7801    7552        -        0
     Init   8013    7897        -        0

>3482964        /34611

    len =    1611   nex =       7

     Term   7020    6595        -        0
     Intr   7158    7104        -        0
     Intr   7297    7245        -        0
     Intr   7457    7381        -        0
     Intr   7801    7552        -        0
     Intr   8050    7897        -        0
     Init   8205    8138        -        0

>3482964        /11428

    len =    2668   nex =       8

     Term   7020    6610        -        0
     Intr   7158    7104        -        0
     Intr   7297    7245        -        0
     Intr   7457    7381        -        0
     Intr   7801    7552        -        0
     Intr   8050    7897        -        0
     Intr   8246    8138        -        0
     Init   8481    8418        -        0
```

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```
>3492855        /38337

   len =    4990   nex =      16

    Init  25848   25975         +        0
    Intr  26062   26140         +        0
    Intr  26270   26331         +        0
    Intr  26602   26716         +        0
    Intr  26811   26886         +        0
    Intr  26966   27061         +        0
    Intr  27278   27322         +        0
    Intr  27671   27727         +        0
    Intr  27827   27982         +        0
    Intr  28080   28145         +        0
    Intr  28647   28770         +        0
    Intr  29541   29654         +        0
    Intr  29769   29846         +        0
    Intr  30008   30055         +        0
    Intr  30266   30334         +        0
    Term  30495   30835         +        0

>3492855        /15984

   len =     850   nex =       0

>3492855        /31772

   len =     380   nex =       1

    Sngl  67588   67967         +        0

>3492855        /42247

   len =     502   nex =       1

    Sngl  67588   68089         +        0

>3492855        /94363

   len =     388   nex =       1

    Sngl  67594   67981         +        0

>3492855        /12727

   len =     698   nex =       1

    Sngl  67594   68291         +        0

>3492855        /5339

   len =     262   nex =       1

    Sngl  67599   67860         +        0

>3492855        /8572

   len =     337   nex =       1

    Sngl  67621   67957         +        0
```

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```
>3492855        /34643

    len =      692    nex =        1

      Sngl   67621   68312          +         0

>3492855        /8142

    len =      698    nex =        1

      Sngl   67639   68336          +         0

>3492855        /113357

    len =      351    nex =        1

      Sngl   67958   68308          +         0

>3510247        /105240

    len =      670    nex =        1

      Sngl    3626    4289          +         0

>3510247        /142022

    len =      226    nex =        1

      Sngl   39517   39742          +         0

>3510247        /26967

    len =     1512    nex =        6

      Init   42812   43093          +         0
      Intr   43184   43305          +         0
      Intr   43393   43479          +         0
      Intr   43565   43627          +         0
      Intr   43886   43923          +         0
      Term   44021   44323          +         0

>3510247        /26134

    len =      574    nex =        3

      Init   42884   43093          +         0
      Intr   43184   43305          +         0
      Term   43393   43457          +         0

>3510336        /38743

    len =     2275    nex =        7

      Term   10758   10440          -         0
      Intr   11079   10844          -         0
      Intr   11501   11396          -         0
      Intr   11676   11590          -         0
      Intr   12031   11813          -         0
      Intr   12289   12119          -         0
```

```
                                              1200
       Init    12714    12390          -          0

    >3510336        /29476

       len =     610    nex =         2

       Init    23119    23477          +          0
       Term    23566    23719          +          0

    >3510336        /18108

       len =     762    nex =         2

       Term    40411    40034          -          0
       Init    40795    40541          -          0

    >3510337        /21311

       len =    3939    nex =         6

       Init     1201     1383          +          0
       Intr     1756     1821          +          0
       Intr     3560     4202          +          0
       Intr     4287     4390          +          0
       Intr     4470     4608          +          0
       Term     4752     5139          +          0

    >3510338        /26524

       len =     686    nex =         1

       Sngl     7586     8271          +          0

    >3510339        /36971

       len =    2077    nex =         7

       Term    13327    13214          -          0
       Intr    13556    13446          -          0
       Intr    13746    13663          -          0
       Intr    13906    13838          -          0
       Intr    14081    14016          -          0
       Intr    14316    14191          -          0
       Init    14804    14412          -          0

    >3510339        /26265

       len =     310    nex =         1

       Sngl    18302    17998          -          0

    >3510339        /3732

       len =     832    nex =         1

       Sngl    18846    18015          -          0

    >3510339        /13162

       len =    1665    nex =         4
```

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```
        Init    25257    25329         +        0
        Intr    25742    25813         +        0
        Intr    26485    26565         +        0
        Term    26649    26921         +        0

>3510339        /101308

     len =      1002    nex =        3

        Init    27964    28382         +        0
        Intr    28442    28624         +        0
        Term    28735    28965         +        0

>3510339        /36973

     len =      4244    nex =       17

        Init    32101    32339         +        0
        Intr    32424    32541         +        0
        Intr    32620    32769         +        0
        Intr    33398    33508         +        0
        Intr    33677    33760         +        0
        Intr    34028    34126         +        0
        Intr    34225    34275         +        0
        Intr    34363    34412         +        0
        Intr    34484    34565         +        0
        Intr    34705    34799         +        0
        Intr    34944    35010         +        0
        Intr    35123    35173         +        0
        Intr    35275    35370         +        0
        Intr    35459    35557         +        0
        Intr    35654    35683         +        0
        Intr    35780    35845         +        0
        Term    36039    36344         +        0

>3510339        /6182

     len =      1225    nex =        6

        Init    35127    35173         +        0
        Intr    35275    35370         +        0
        Intr    35459    35557         +        0
        Intr    35654    35683         +        0
        Intr    35780    35845         +        0
        Term    36039    36351         +        0

>3510339        /7341

     len =      1630    nex =        5

        Term     2222     1970         -        0
        Intr     2399     2316         -        0
        Intr     2651     2492         -        0
        Intr     3073     3025         -        0
        Init     3595     3310         -        0

>3510339        /37148

     len =      1831    nex =        3
     Init   36683   36792         +        0
     Intr   36895   36996         +        0
     Term   37400   38513         +        0

>3510339       /4190

   len =      570   nex =        1

     Sngl   37975   38544         +        0

>3510339       /27885

   len =      910   nex =        4

     Init   47055   47312         +        0
     Intr   47405   47463         +        0
     Intr   47558   47675         +        0
     Term   47755   47961         +        0

>3510339       /32353

   len =     1336   nex =        5

     Init   53149   53304         +        0
     Intr   53397   53504         +        0
     Intr   53574   53700         +        0
     Intr   53970   54038         +        0
     Term   54133   54484         +        0

>3510339       /14885

   len =      688   nex =        1

     Sngl    8005    7318         -        0

>3510340       /126460

   len =      934   nex =        2

     Term   21669   21324         -        0
     Init   22257   21750         -        0

>3510340       /40096

   len =     2290   nex =        7

     Term   38031   37656         -        0
     Intr   38245   38120         -        0
     Intr   38564   38358         -        0
     Intr   38726   38658         -        0
     Intr   38905   38813         -        0
     Intr   39170   38997         -        0
     Init   39945   39536         -        0

>3510340       /14006

   len =     3759   nex =       18

     Term   41382   41098         -        0
     Intr   41552   41486        -        0
     Intr   41808   41760        -        0
     Intr   42179   42123        -        0
     Intr   42406   42335        -        0
     Intr   42585   42494        -        0
     Intr   42747   42675        -        0
     Intr   42944   42870        -        0
     Intr   43109   43045        -        0
     Intr   43297   43210        -        0
     Intr   43431   43387        -        0
     Intr   43733   43614        -        0
     Intr   43878   43840        -        0
     Intr   44054   43971        -        0
     Intr   44229   44138        -        0
     Intr   44386   44312        -        0
     Intr   44577   44484        -        0
     Init   44856   44655        -        0

>3510341       /10879

    len =     1930    nex =       0

>3510341       /36005

    len =      691    nex =       1

     Sngl   40942   41632        +        0

>3510341       /32092

    len =     2099    nex =       9

     Term   48160   47836        -        0
     Intr   48310   48244        -        0
     Intr   48500   48383        -        0
     Intr   48656   48600        -        0
     Intr   48836   48741        -        0
     Intr   49030   48923        -        0
     Intr   49183   49114        -        0
     Intr   49608   49481        -        0
     Init   49934   49746        -        0

>3510341       /18565

    len =     1332    nex =       3

     Term   55802   55320        -        0
     Intr   56319   56196        -        0
     Init   56651   56408        -        0

>3510342       /21714

    len =     1319    nex =       1

     Sngl   54886   56204        +        0

>3510342       /24640

    len =      730    nex =       3
```

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```
     Init   56678   56815         +        0
     Intr   56912   56980         +        0
     Term   57073   57398         +        0

>3510342        /123863

    len =     910   nex =        1

     Sngl   60334   61240         +        0

>3510342        /534

    len =     536   nex =        1

     Sngl    9075    8540         -        0

>3510343        /40742

    len =    1891   nex =        7

     Init   13415   13697         +        0
     Intr   13866   14012         +        0
     Intr   14098   14196         +        0
     Intr   14285   14476         +        0
     Intr   14612   14731         +        0
     Intr   14818   14889         +        0
     Term   14970   15305         +        0

>3510343        /24171

    len =    1330   nex =        1

     Sngl    2256    3585         +        0

>3510343        /2722

    len =     503   nex =        1

     Sngl   30214   29712         -        0

>3510343        /106836

    len =    1702   nex =        9

     Term   33894   33628         -        0
     Intr   34089   33998         -        0
     Intr   34258   34188         -        0
     Intr   34371   34329         -        0
     Intr   34518   34462         -        0
     Intr   34704   34605         -        0
     Intr   34927   34777         -        0
     Intr   35103   35021         -        0
     Init   35329   35222         -        0

>3510343        /6903

    len =    1763   nex =        4

     Init   37722   38574         +        0
     Intr   38655   38839         +        0
```

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```
     Intr   38929   39039          +        0
     Term   39124   39484          +        0

>3510343       /347

    len =     1651   nex =       3

     Init    4180    4489          +        0
     Intr    5552    5687          +        0
     Term    5778    5830          +        0

>3510343       /97314

    len =     1179   nex =       3

     Term   41393   41220          -        0
     Intr   41794   41558          -        0
     Init   42398   42147          -        0

>3510343       /29713

    len =      614   nex =       1

     Sngl   43022   42748          -        0

>3510343       /42932

    len =     3299   nex =      12

     Term   44028   43691          -        0
     Intr   44238   44168          -        0
     Intr   44426   44307          -        0
     Intr   44873   44679          -        0
     Intr   45135   45071          -        0
     Intr   45321   45231          -        0
     Intr   45556   45495          -        0
     Intr   45732   45682          -        0
     Intr   45918   45817          -        0
     Intr   46113   46006          -        0
     Intr   46273   46210          -        0
     Init   46989   46641          -        0

>3510343       /27758

    len =     1726   nex =       1

     Sngl   48931   50656          +        0

>3510343       /41873

    len =     1120   nex =       1

     Sngl   49483   50602          +        0

>3510343       /103302

    len =      346   nex =       1

     Sngl   50315   50660          +        0
```

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```
>3510343        /11988

   len =    2000   nex =       1

     Sngl   53425   52376        -        0

>3510343        /40911

   len =     533   nex =       2

     Term   54279   53987        -        0
     Init   54519   54367        -        0

>3510343        /4645

   len =    2130   nex =       6

     Init   68383   68789        +        0
     Intr   69020   69154        +        0
     Intr   69249   69327        +        0
     Intr   69422   69516        +        0
     Intr   69735   69902        +        0
     Term   69982   70512        +        0

>3510343        /40013

   len =     219   nex =       1

     Sngl   77830   77612        -        0

>3510343        /4267

   len =     790   nex =       1

     Sngl   82643   83428        +        0

>3510344        /2679

   len =     511   nex =       1

     Sngl   43185   43695        +        0

>3510345        /38935

   len =    1870   nex =       5

     Term   20537   19806        -        0
     Intr   20684   20623        -        0
     Intr   20927   20816        -        0
     Intr   21079   21017        -        0
     Init   21667   21540        -        0

>3510345        /16321

   len =    1839   nex =       6

     Init   30949   31052        +        0
     Intr   31463   31499        +        0
     Intr   31947   32020        +        0
     Intr   32124   32217        +        0
```

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```
     Intr   32330   32412         +        0
     Term   32494   32787         +        0

>3510345       /9887

    len =     1957   nex =       5

     Init   31463   31499         +        0
     Intr   31947   32020         +        0
     Intr   32124   32217         +        0
     Intr   32330   32412         +        0
     Term   32494   32923         +        0

>3510345       /40590

    len =     2650   nex =       7

     Init   36093   36290         +        0
     Intr   36588   37160         +        0
     Intr   37250   37316         +        0
     Intr   37733   37824         +        0
     Intr   37917   38126         +        0
     Intr   38223   38360         +        0
     Term   38470   38726         +        0

>3510345       /42141

    len =     3010   nex =      12

     Init   61602   61918         +        0
     Intr   62116   62244         +        0
     Intr   62321   62413         +        0
     Intr   62503   62582         +        0
     Intr   62663   62776         +        0
     Intr   62944   63017         +        0
     Intr   63156   63232         +        0
     Intr   63328   63411         +        0
     Intr   63507   63599         +        0
     Intr   63687   63794         +        0
     Intr   63877   63948         +        0
     Term   64039   64603         +        0

>3510345       /21808

    len =     3836   nex =       4

     Term   82161   81264         -        0
     Intr   82858   82787         -        0
     Intr   83396   83347         -        0
     Init   84608   84277         -        0

>3510346       /34019

    len =      465   nex =       1

     Sngl   19046   18582         -        0

>3510346       /3859

    len =      583   nex =       1
```

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```
     Sngl   19162   18580        -       0

>3510346       /41105

    len =    2655   nex =       4

     Term   21838   21322        -       0
     Intr   22141   21929        -       0
     Intr   22425   22322        -       0
     Init   23176   22513        -       0

>3510346       /18886

    len =    1400   nex =       1

     Sngl    2565    1166        -       0

>3510346       /6830

    len =    1411   nex =       2

     Init   28915   29605        +       0
     Term   29936   30325        +       0

>3510346       /19036

    len =     925   nex =       2

     Init   29413   29605        +       0
     Term   29936   30323        +       0

>3510347       /32287

    len =    1572   nex =       6

     Term   17536   17338        -       0
     Intr   17706   17628        -       0
     Intr   17947   17881        -       0
     Intr   18159   18060        -       0
     Intr   18386   18246        -       0
     Init   18909   18664        -       0

>3510347       /10832

    len =    1590   nex =       5

     Term   17536   17344        -       0
     Intr   17706   17628        -       0
     Intr   17947   17881        -       0
     Intr   18159   18060        -       0
     Init   18386   18246        -       0

>3510347       /8265

    len =    1462   nex =       1

     Sngl   25560   25814        +       0

>3510347       /37190
```

```
    len =    2683   nex =        8

     Init   26268   26469          +        0
     Intr   27118   27309          +        0
     Intr   27478   27554          +        0
     Intr   27755   27814          +        0
     Intr   27939   28142          +        0
     Intr   28258   28320          +        0
     Intr   28413   28481          +        0
     Term   28719   28950          +        0

>3510347        /21563

    len =    1497   nex =        6

     Term   32966   32764          -        0
     Intr   33122   33065          -        0
     Intr   33434   33389          -        0
     Intr   33570   33535          -        0
     Intr   33921   33809          -        0
     Init   34260   34059          -        0

>3510347        /6

    len =    2248   nex =        8

     Term   35112   34642          -        0
     Intr   35312   35190          -        0
     Intr   35520   35412          -        0
     Intr   35687   35606          -        0
     Intr   36105   35898          -        0
     Intr   36365   36207          -        0
     Intr   36591   36457          -        0
     Init   36889   36687          -        0

>3510347        /40282

    len =    1030   nex =        1

     Sngl   44145   43121          -        0

>3510347        /21002

    len =    1112   nex =        4

     Init   44576   44637          +        0
     Intr   44840   44941          +        0
     Intr   45017   45138          +        0
     Term   45230   45687          +        0

>3510347        /8259

    len =     911   nex =        2

     Init   46905   47248          +        0
     Term   47327   47815          +        0

>3510347        /101505
```

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```
     len =     1259    nex =        5

      Init   49449   49599          +        0
      Intr   49835   50106          +        0
      Intr   50195   50285          +        0
      Intr   50364   50430          +        0
      Term   50515   50707          +        0

>3510347        /2528

     len =     1377    nex =        2

      Term   57801   57178          -        0
      Init   58554   58264          -        0

>3510347        /112017

     len =      914    nex =        2

      Term   57801   57641          -        0
      Init   58554   58264          -        0

>3510347        /21882

     len =     2650    nex =        3

      Init   60423   61009          +        0
      Intr   61588   61725          +        0
      Term   62599   63070          +        0

>3510347        /93510

     len =     1477    nex =        3

      Init   64300   64751          +        0
      Intr   64887   65024          +        0
      Term   65353   65776          +        0

>3510347        /25205

     len =     2153    nex =        5

      Init   68370   68963          +        0
      Intr   69054   69194          +        0
      Intr   69282   69422          +        0
      Intr   69950   70056          +        0
      Term   70149   70522          +        0

>3510347        /10

     len =      731    nex =        2

      Term    6348     6208          -        0
      Init    6938     6424          -        0

>3510347        /20808

     len =     1651    nex =        4

      Init   72240   72762          +        0
```

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```
      Intr   72870   73010          +        0
      Intr   73158   73298          +        0
      Term   73581   73890          +        0

>3510347       /3268

     len =     670   nex =       2

      Term   74338   73874          -        0
      Init   74537   74427          -        0

>3510347       /34224

     len =    2512   nex =       6

      Init    8462    8802          +        0
      Intr    9019    9104          +        0
      Intr    9299    9349          +        0
      Intr   10221   10287          +        0
      Intr   10414   10493          +        0
      Term   10655   10973          +        0

>3513725       /36053

     len =    1750   nex =       7

      Init   13019   13159          +        0
      Intr   13469   13609          +        0
      Intr   13779   13816          +        0
      Intr   13897   13947          +        0
      Intr   14038   14205          +        0
      Intr   14282   14394          +        0
      Term   14473   14764          +        0

>3513725       /93362

     len =    2622   nex =       6

      Init   25174   25560          +        0
      Intr   25648   25712          +        0
      Intr   25798   25884          +        0
      Intr   25990   26129          +        0
      Intr   27220   27274          +        0
      Term   27422   27795          +        0

>3513725       /30013

     len =    2650   nex =       6

      Init   25215   25560          +        0
      Intr   25648   25712          +        0
      Intr   25798   25884          +        0
      Intr   25990   26129          +        0
      Intr   27220   27274          +        0
      Term   27422   27859          +        0

>3513725       /15608

     len =    2626   nex =       9
```

```
         Init   28826   29089          +        0
         Intr   29751   29800          +        0
         Intr   29928   30063          +        0
         Intr   30157   30258          +        0
         Intr   30340   30567          +        0
         Intr   30642   30720          +        0
         Intr   30807   30889          +        0
         Intr   30975   31066          +        0
         Term   31194   31451          +        0

>3513725        /11425

      len =     1812    nex =        3

         Init   43000   43341          +        0
         Intr   43510   43706          +        0
         Term   44140   44588          +        0

>3513725        /4588

      len =     1847    nex =        3

         Init   42785   43341          +        0
         Intr   43510   43706          +        0
         Term   44140   44631          +        0

>3513725        /19449

      len =     1469    nex =        3

         Init   48183   48572          +        0
         Intr   48751   48856          +        0
         Term   49391   49651          +        0

>3513725        /33161

      len =     1990    nex =        5

         Term   70844   70396          -        0
         Intr   71145   71026          -        0
         Intr   71729   71348          -        0
         Intr   72167   72131          -        0
         Init   72376   72258          -        0

>3513725        /2538

      len =     2710    nex =        4

         Term   88530   88065          -        0
         Intr   88919   88628          -        0
         Intr   89919   89306          -        0
         Init   90771   90027          -        0

>3522932        /1233

      len =      468    nex =        1

         Sngl   14882   14415          -        0

>3522932        /6247
```

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```
    len =     629    nex =        1

     Sngl   14910   14282          -        0

>3522932        /34004

    len =     670    nex =        1

     Sngl   14939   14277          -        0

>3522932        /34114

    len =     970    nex =        1

     Sngl   26932   27893          +        0

>3522932        /119203

    len =     310    nex =        1

     Sngl   31113   31414          +        0

>3522932        /40043

    len =    2092    nex =        2

     Init   33181   34387          +        0
     Term   34475   35272          +        0

>3522932        /17422

    len =    1694    nex =        8

     Term   85840   85605          -        0
     Intr   85984   85923          -        0
     Intr   86150   86064          -        0
     Intr   86316   86236          -        0
     Intr   86490   86395          -        0
     Intr   86710   86576          -        0
     Intr   86925   86803          -        0
     Init   87172   87000          -        0

>3548797        /39349

    len =    1390    nex =        1

     Sngl   45745   47127          +        0

>3548797        /118505

    len =    1236    nex =        5

     Term   54662   54394          -        0
     Intr   54808   54746          -        0
     Intr   54939   54898          -        0
     Intr   55250   55035          -        0
     Init   55629   55345          -        0

>3548797        /37655
```

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```
        len =     2848    nex =       11

         Term   73586   73293          -          0
         Intr   73848   73771          -          0
         Intr   74084   73948          -          0
         Intr   74268   74199          -          0
         Intr   74454   74368          -          0
         Intr   74621   74571          -          0
         Intr   74888   74761          -          0
         Intr   75233   75148          -          0
         Intr   75468   75317          -          0
         Intr   75678   75563          -          0
         Init   76140   75987          -          0

   >3582315        /29796

        len =     1556    nex =        1

         Sngl   45020   43465          -          0

   >3582315        /34214

        len =     1941    nex =        6

         Init   46259   46498          +          0
         Intr   46588   46734          +          0
         Intr   46826   46878          +          0
         Intr   46958   47120          +          0
         Intr   47207   47435          +          0
         Term   47518   48199          +          0

   >3582315        /12086

        len =      651    nex =        1

         Sngl   51096   50446          -          0

   >3582315        /36220

        len =     1908    nex =        2

         Init   64549   65215          +          0
         Term   66042   66456          +          0

   >3600029        /26159

        len =      390    nex =        1

         Sngl   24328   23939          -          0

   >3600029        /35159

        len =     1179    nex =        1

         Sngl   25177   23999          -          0

   >3600045        /24130

        len =     1030    nex =        3
```

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```
     Term   15293   14783         -        0
     Intr   15574   15428         -        0
     Init   15807   15656         -        0

>3600045        /32602

    len =     1450   nex =        1

     Sngl   25383   26825         +        0

>3600045        /25988

    len =      824   nex =        2

     Term    3124    2675         -        0
     Init    3498    3344         -        0

>3600045        /22850

    len =      408   nex =        2

     Term   41689   41456         -        0
     Init   41863   41773         -        0

>3600045        /35662

    len =     1570   nex =        3

     Term   51918   51401         -        0
     Intr   52566   51996         -        0
     Init   52969   52885         -        0

>3608126        /5901

    len =     1296   nex =        3

     Term   11196   10881         -        0
     Intr   11486   11310         -        0
     Init   12176   11888         -        0

>3608126        /3992

    len =     1349   nex =        5

     Term   26268   26001         -        0
     Intr   26428   26385         -        0
     Intr   26650   26563         -        0
     Intr   27078   27031         -        0
     Init   27343   27172         -        0

>3608126        /9670

    len =     1059   nex =        2

     Term    1939    1719         -        0
     Init    2777    2477         -        0

>3608126        /17424
```

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```
    len =     2011    nex =        8

     Term   33042   32798          -         0
     Intr   33307   33131          -         0
     Intr   33518   33400          -         0
     Intr   33658   33592          -         0
     Intr   33803   33742          -         0
     Intr   34105   33911          -         0
     Intr   34389   34199          -         0
     Init   34808   34562          -         0

>3608126        /38584

    len =     1669    nex =        3

     Init   34965   35667          +         0
     Intr   35839   36034          +         0
     Term   36222   36633          +         0

>3608126        /13821

    len =      873    nex =        3

     Init   39040   39422          +         0
     Intr   39532   39576          +         0
     Term   39671   39912          +         0

>3608126        /36332

    len =     3702    nex =        6

     Init    4194    4297          +         0
     Intr    5595    5680          +         0
     Intr    6594    6973          +         0
     Intr    7084    7213          +         0
     Intr    7302    7588          +         0
     Term    7672    7895          +         0

>3608126        /23118

    len =      814    nex =        4

     Term   56920   56791          -         0
     Intr   57180   57010          -         0
     Intr   57415   57327          -         0
     Init   57604   57495          -         0

>3608126        /24194

    len =     1115    nex =        4

     Term   56920   56626          -         0
     Intr   57180   57010          -         0
     Intr   57415   57327          -         0
     Init   57740   57495          -         0

>3617740        /23672

    len =     2710    nex =        6
```

```
                                         1217
      Term      779       523        -         0
      Intr      995       869        -         0
      Intr     1176      1079        -         0
      Intr     1629      1462        -         0
      Intr     2092      1983        -         0
      Init     2233      2181        -         0

 >3617740        /14534

    len =     2050    nex =        5

      Init    38982     39082        +         0
      Intr    39269     39379        +         0
      Intr    39472     39776        +         0
      Intr    39865     40274        +         0
      Term    40379     40796        +         0

 >3617740        /17803

    len =      878    nex =        4

      Init    38750     38796        +         0
      Intr    38982     39082        +         0
      Intr    39269     39379        +         0
      Term    39472     39614        +         0

 >3617740        /125212

    len =      613    nex =        2

      Init    40232     40274        +         0
      Term    40379     40844        +         0

 >3617740        /4609

    len =     3370    nex =       14

      Term    47827     47264        -         0
      Intr    48084     47941        -         0
      Intr    48277     48170        -         0
      Intr    48449     48436        -         0
      Intr    48556     48515        -         0
      Intr    48691     48640        -         0
      Intr    48810     48769        -         0
      Intr    48971     48922        -         0
      Intr    49144     49080        -         0
      Intr    49457     49334        -         0
      Intr    49729     49648        -         0
      Intr    49877     49801        -         0
      Intr    50044     49973        -         0
      Init    50199     50135        -         0

 >3641835        /124015

    len =      586    nex =        2

      Term   104751    104680        -         0
      Init   105265    105103        -         0

 >3641835        /24998
```

```
     len =    1358    nex =       3

       Term  104368  103951           -         0
       Intr  104751  104466           -         0
       Init  105308  105103           -         0

>3641835        /40208

     len =    1313    nex =       3

       Term  106166  106021           -         0
       Intr  106634  106461           -         0
       Init  107333  106949           -         0

>3641835        /10510

     len =     692    nex =       1

       Sngl  118544  117853           -         0

>3641835        /29665

     len =    2207    nex =       6

       Term   13437   13279           -         0
       Intr   13643   13530           -         0
       Intr   14090   13733           -         0
       Intr   14763   14339           -         0
       Intr   15097   15003           -         0
       Init   15485   15170           -         0

>3641835        /101430

     len =     600    nex =       3

       Term   19477   19405           -         0
       Intr   19845   19550           -         0
       Init   20004   19937           -         0

>3641835        /8586

     len =    1191    nex =       3

       Term   40504   40292           -         0
       Intr   41034   40900           -         0
       Init   41253   41179           -         0

>3641835        /40231

     len =    1140    nex =       1

       Sngl   57915   59054           +         0

>3641835        /6475

     len =     460    nex =       1

       Sngl   58753   59203           +         0
```

```
>3641835       /42589

   len =    1193   nex =       3

    Term   71259   70935         -         0
    Intr   71875   71830         -         0
    Init   72127   71973         -         0

>3641835       /29919

   len =    1271   nex =       3

    Term   71259   70897         -         0
    Intr   71875   71830         -         0
    Init   72167   71973         -         0

>3641835       /27483

   len =     833   nex =       1

    Sngl   73741   72909         -         0

>3641835       /20883

   len =     797   nex =       1

    Sngl   73759   72963         -         0

>3641835       /28635

   len =    3595   nex =      12

    Init   74327   74696         +         0
    Intr   75055   75097         +         0
    Intr   75428   75503         +         0
    Intr   75665   75734         +         0
    Intr   76009   76158         +         0
    Intr   76267   76371         +         0
    Intr   76474   76620         +         0
    Intr   76796   76871         +         0
    Intr   77032   77120         +         0
    Intr   77218   77309         +         0
    Intr   77476   77518         +         0
    Term   77612   77921         +         0

>3641835       /37733

   len =    2501   nex =      13

    Init   78502   78584         +         0
    Intr   78669   78711         +         0
    Intr   78845   78934         +         0
    Intr   79022   79097         +         0
    Intr   79225   79294         +         0
    Intr   79399   79548         +         0
    Intr   79650   79754         +         0
    Intr   79835   79981         +         0
    Intr   80095   80170         +         0
    Intr   80300   80388         +         0
    Intr   80465   80556         +         0
```

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```
    Intr   80724   80766         +        0
    Term   80863   80994         +        0

>3641835       /32357

  len =    3190   nex =      10

    Term   81717   81263         -        0
    Intr   81922   81848         -        0
    Intr   82305   82153         -        0
    Intr   82488   82426         -        0
    Intr   82779   82579         -        0
    Intr   83016   82915         -        0
    Intr   83421   83270         -        0
    Intr   83600   83531         -        0
    Intr   83792   83679         -        0
    Init   84448   84248         -        0

>3641835       /143247

  len =    1363   nex =       5

    Init   85011   85116         +        0
    Intr   85354   85491         +        0
    Intr   85562   85747         +        0
    Intr   85840   86040         +        0
    Term   86137   86234         +        0

>3641835       /24125

  len =     578   nex =       2

    Init   85840   86040         +        0
    Term   86137   86267         +        0

>3641835       /27838

  len =    1964   nex =       4

    Init   92854   93018         +        0
    Intr   93122   93163         +        0
    Intr   93954   94035         +        0
    Term   94181   94236         +        0

>3641835       /18715

  len =    2001   nex =       4

    Init   92859   93018         +        0
    Intr   93122   93163         +        0
    Intr   93954   94035         +        0
    Term   94181   94236         +        0

>3641835       /22619

  len =     837   nex =       1

    Sngl   97080   96244         -        0

>3643588       /7152
```

```
     len =      700    nex =        1

      Sngl  100213  100912         +        0

>3643588        /26655

     len =     2932    nex =       10

      Init   23965   24029         +        0
      Intr   24558   24733         +        0
      Intr   24818   24949         +        0
      Intr   25037   25087         +        0
      Intr   25184   25280         +        0
      Intr   25536   25582         +        0
      Intr   25690   25909         +        0
      Intr   26128   26257         +        0
      Intr   26350   26392         +        0
      Term   26519   26896         +        0

>3643588        /39893

     len =     1292    nex =        1

      Sngl   34172   32881         -        0

>3643588        /33811

     len =      619    nex =        1

      Sngl   38584   37966         -        0

>3643588        /14229

     len =       88    nex =        1

      Sngl   44849   44762         -        0

>3643588        /107487

     len =      688    nex =        1

      Sngl   91073   91760         +        0

>3650026        /105053

     len =     1096    nex =        1

      Sngl   15351   15025         -        0

>3650026        /41621

     len =     1450    nex =        2

      Term   14294   13985         -        0
      Init   15430   15025         -        0

>3650026        /98613

     len =      103    nex =        1
```

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```
       Sngl    20145    20247          +         0

    >3650026        /1845

      len =     1078    nex =        1

       Sngl    25288    25744          +         0

    >3650026        /105106

      len =      430    nex =        1

       Sngl    25301    25714          +         0

    >3650026        /18430

      len =     2276    nex =        5

       Term    27554    27148          -         0
       Intr    27977    27733          -         0
       Intr    28182    28075          -         0
       Intr    28573    28309          -         0
       Init    29423    29071          -         0

    >3659491        /25383

      len =     4510    nex =       13

       Term     9461     9027          -         0
       Intr     9645     9554          -         0
       Intr     9862     9749          -         0
       Intr    10492    10129          -         0
       Intr    10829    10696          -         0
       Intr    11104    10996          -         0
       Intr    11365    11244          -         0
       Intr    11551    11442          -         0
       Intr    11745    11641          -         0
       Intr    12001    11843          -         0
       Intr    12392    12337          -         0
       Intr    12653    12602          -         0
       Init    13533    13069          -         0

    >3659491        /40893

      len =     1515    nex =        4

       Init    17945    18140          +         0
       Intr    18495    18596          +         0
       Intr    18711    18833          +         0
       Term    19154    19459          +         0

    >3659491        /11713

      len =      647    nex =        1

       Sngl    29537    28891          -         0

    >3659491        /1822
```

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```
len =     1542    nex =       4

 Term   30282    29975         -         0
 Intr   30669    30547         -         0
 Intr   30870    30769         -         0
 Init   31516    31375         -         0

>3659491        /16879

len =      792    nex =       2

 Term   43340    43008         -         0
 Init   43799    43512         -         0

>3659491        /31696

len =     1488    nex =       4

 Init   52934    53129         +         0
 Intr   53228    53290         +         0
 Intr   54065    54124         +         0
 Term   54224    54421         +         0

>3659491        /3853

len =     1674    nex =       3

 Init   67957    68461         +         0
 Intr   68545    68737         +         0
 Term   69218    69630         +         0

>3659491        /22630

len =     1510    nex =       3

 Init   67989    68461         +         0
 Intr   68545    68737         +         0
 Term   69218    69495         +         0

>3659491        /119226

len =     1460    nex =       1

 Sngl   80172    80692         +         0

>3659491        /36272

len =     1398    nex =       1

 Sngl   80172    80692         +         0

>3659491        /31768

len =     1795    nex =       3

 Init   82901    82956         +         0
 Intr   83626    83993         +         0
 Term   84259    84695         +         0

>3659491        /1530
```

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```
  len =    1113   nex =       2

   Term   86548   85965          -        0
   Init   87077   86859          -        0

>3659491        /17503

  len =     270   nex =       1

   Sngl    9313    9044          -        0

>3668073        /16622

  len =    1358   nex =       4

   Init   23250   23680          +        0
   Intr   23767   23864          +        0
   Intr   23960   24025          +        0
   Term   24310   24607          +        0

>3668073        /37897

  len =    1111   nex =       1

   Sngl   51343   52453          +        0

>3668073        /37026

  len =    1930   nex =       6

   Init     694     789          +        0
   Intr    1152    1397          +        0
   Intr    1494    1661          +        0
   Intr    1741    1834          +        0
   Intr    1915    2085          +        0
   Term    2221    2618          +        0

>3687221        /16515

  len =    1589   nex =       3

   Init  102216  102407          +        0
   Intr  102664  102801          +        0
   Term  103376  103791          +        0

>3687221        /9466

  len =    1096   nex =       3

   Term  107635  107345          -        0
   Intr  108241  108104          -        0
   Init  108440  108355          -        0

>3687221        /14718

  len =    1498   nex =       1

   Sngl  121547  121349          -        0
```

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```
>3687221        /15861

   len =      973   nex =        5

    Init   91525   91626          +         0
    Intr   91730   91799          +         0
    Intr   91913   91991          +         0
    Intr   92096   92177          +         0
    Term   92263   92497          +         0

>3687221        /5064

   len =     1290   nex =        4

    Term   95134   94875          -         0
    Intr   95305   95237          -         0
    Intr   95573   95487          -         0
    Init   96164   95953          -         0

>3688169        /36835

   len =     2308   nex =        8

    Term   10054    9794          -         0
    Intr   10172   10133          -         0
    Intr   10479   10407          -         0
    Intr   10683   10607          -         0
    Intr   10809   10773          -         0
    Intr   10942   10882          -         0
    Intr   11425   11225          -         0
    Init   12081   11998          -         0

>3688169        /39326

   len =     1424   nex =        4

    Init   16580   16851          +         0
    Intr   16934   16974          +         0
    Intr   17077   17132          +         0
    Term   17802   18003          +         0

>3688169        /8739

   len =     1450   nex =        4

    Init   29897   30039          +         0
    Intr   30457   30564          +         0
    Intr   30834   31001          +         0
    Term   31120   31345          +         0

>3688169        /95066

   len =      850   nex =        1

    Sngl    6958    7799          +         0

>3688169        /4868

   len =      590   nex =        1
```

```
     Sngl   72372   71783          -         0

>3695372        /25253

    len =     2094   nex =        11

     Init     1783     2087         +         0
     Intr     2177     2257         +         0
     Intr     2345     2440         +         0
     Intr     2533     2616         +         0
     Intr     2705     2774         +         0
     Intr     2854     2936         +         0
     Intr     3017     3096         +         0
     Intr     3178     3276         +         0
     Intr     3365     3434         +         0
     Intr     3510     3599         +         0
     Term     3708     3876         +         0

>3695372        /20974

    len =     9399   nex =         1

     Sngl   20229   19834          -         0

>3695386        /24263

    len =      711   nex =         1

     Sngl   19462   20172          +         0

>3695400        /34605

    len =      462   nex =         1

     Sngl   10570   11031          +         0

>3695400        /1489

    len =     1587   nex =         2

     Term   15637   15331          -         0
     Init   16917   16616          -         0

>3695400        /10189

    len =      985   nex =         2

     Init   25271   25588          +         0
     Term   25943   26252          +         0

>3695400        /1831

    len =      477   nex =         1

     Sngl   26645   26169          -         0

>3695400        /35152

    len =      455   nex =         1
```

```
                                      1227
   Sngl    27846   28300        +        0

>3695400        /108385

    len =      629   nex =       3

   Init     3404    3506        +        0
   Intr     3589    3680        +        0
   Term     3749    4032        +        0

>3695400        /2125

    len =     1522   nex =       2

   Term     5925    5502        -        0
   Init     7023    6790        -        0

>3695400        /28203

    len =     2144   nex =       5

   Term    77122   76779        -        0
   Intr    77397   77265        -        0
   Intr    77620   77515        -        0
   Intr    77799   77729        -        0
   Init    78922   78765        -        0

>3702315        /32891

    len =     2096   nex =       9

   Init    15705   15815        +        0
   Intr    15919   16138        +        0
   Intr    16274   16489        +        0
   Intr    16586   16719        +        0
   Intr    16845   16916        +        0
   Intr    17042   17131        +        0
   Intr    17227   17313        +        0
   Intr    17401   17480        +        0
   Term    17583   17792        +        0

>3702315        /34383

    len =     2082   nex =       7

   Init    18147   18358        +        0
   Intr    18507   18722        +        0
   Intr    18818   18951        +        0
   Intr    19279   19368        +        0
   Intr    19462   19548        +        0
   Intr    19649   19728        +        0
   Term    19837   20078        +        0

>3702315        /38881

    len =     2006   nex =       7

   Init    18051   18358        +        0
   Intr    18507   18722        +        0
   Intr    18818   18951        +        0
```

```
        Intr    19279    19368          +       0
        Intr    19462    19548          +       0
        Intr    19649    19728          +       0
        Term    19837    20056          +       0

>3702315        /126587

      len =     1758    nex =        9

        Init    37610    37788          +       0
        Intr    37865    37924          +       0
        Intr    38002    38107          +       0
        Intr    38260    38302          +       0
        Intr    38382    38454          +       0
        Intr    38543    38630          +       0
        Intr    38770    38826          +       0
        Intr    38917    38995          +       0
        Term    39096    39367          +       0

>3702315        /39624

      len =     1673    nex =        9

        Init    37636    37788          +       0
        Intr    37865    37924          +       0
        Intr    38002    38107          +       0
        Intr    38260    38302          +       0
        Intr    38382    38454          +       0
        Intr    38543    38630          +       0
        Intr    38770    38826          +       0
        Intr    38917    38995          +       0
        Term    39096    39308          +       0

>3702315        /1835

      len =     1311    nex =        8

        Init    37685    37788          +       0
        Intr    37865    37924          +       0
        Intr    38002    38107          +       0
        Intr    38260    38302          +       0
        Intr    38382    38454          +       0
        Intr    38543    38630          +       0
        Intr    38770    38826          +       0
        Term    38917    38989          +       0
>3702315        /13319

      len =     2328    nex =        8

        Term    39649    39295          -       0
        Intr    39862    39780          -       0
        Intr    40012    39960          -       0
        Intr    40165    40081          -       0
        Intr    40534    40435          -       0
        Intr    40776    40626          -       0
        Intr    40939    40857          -       0
        Init    41622    41363          -       0

>3702315        /16400
```

```
     len =      746   nex =        2

      Init    4313    4574          +         0
      Term    4732    5058          +         0

 >3702315        /22834

     len =      711   nex =        2

      Init    4314    4711          +         0
      Term    4732    5024          +         0

 >3702315        /2011

     len =      815   nex =        1

      Sngl   50261   49447          -         0

 >3702315        /150696

     len =      813   nex =        1

      Sngl     805    1395          +         0

 >3702315        /9694

     len =     1390   nex =        2

      Term   63455   63085          -         0
      Init   64470   64155          -         0

 >3702315        /2747

     len =     1450   nex =        2

      Term   63455   63044          -         0
      Init   64488   64155          -         0

 >3702315        /37367

     len =     1800   nex =        6

      Init   75775   75964          +         0
      Intr   76051   76180          +         0
      Intr   76334   76471          +         0
      Intr   76560   76892          +         0
      Intr   76989   77172          +         0
      Term   77268   77574          +         0

 >3702722        /2662

     len =     2085   nex =        9

      Term   14429   14277          -         0
      Intr   14686   14527          -         0
      Intr   14858   14767          -         0
      Intr   15138   14935          -         0
      Intr   15415   15221          -         0
      Intr   15543   15495          -         0
      Intr   15749   15646          -         0
```

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```
                                              1230
        Intr     15877    15838          -         0
        Init     16361    16286          -         0

    >3702722         /34554

       len =      2372    nex =         9

        Term     14429    14066          -         0
        Intr     14686    14527          -         0
        Intr     14858    14767          -         0
        Intr     15138    14935          -         0
        Intr     15415    15221          -         0
        Intr     15543    15495          -         0
        Intr     15749    15646          -         0
        Intr     15877    15838          -         0
        Init     16437    16286          -         0

    >3702722         /14234

       len =      1597    nex =         5

        Term     50617    50334          -         0
        Intr     50852    50691          -         0
        Intr     51106    51000          -         0
        Intr     51288    51207          -         0
        Init     51930    51764          -         0

    >3702722         /35228

       len =      2920    nex =         6

        Term     62691    62388          -         0
        Intr     63047    62898          -         0
        Intr     63282    63083          -         0
        Intr     63460    63392          -         0
        Intr     63685    63542          -         0
        Init     64731    64543          -         0

    >3702722         /150222

       len =       885    nex =         2

        Term     67157    66736          -         0
        Init     67620    67353          -         0

    >3702723         /639

       len =      2568    nex =        11

        Term     33692    33399          -         0
        Intr     33810    33769          -         0
        Intr     34153    34055          -         0
        Intr     34894    34809          -         0
        Intr     35061    34977          -         0
        Intr     35224    35141          -         0
        Intr     35373    35294          -         0
        Intr     35512    35449          -         0
        Intr     35642    35574          -         0
        Intr     35787    35707          -         0
        Init     35966    35863          -         0
```

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```
>3702723        /12930

   len =     673    nex =        2

    Init    9767    9878          +         0
    Term   10190   10439          +         0

>3702724        /20287

   len =    1517    nex =        6

    Init   20008   20168          +         0
    Intr   20256   20311          +         0
    Intr   20582   20705          +         0
    Intr   20799   20905          +         0
    Intr   21133   21196          +         0
    Term   21314   21524          +         0

>3702724        /94685

   len =     259    nex =        1

    Sngl   38461   38203          -         0

>3702724        /33455

   len =    1271    nex =        1

    Sngl   39470   38200          -         0

>3702724        /6342

   len =    2050    nex =        5

    Init   47979   48280          +         0
    Intr   48382   48502          +         0
    Intr   48605   48696          +         0
    Intr   49127   49223          +         0
    Term   49436   50023          +         0

>3702724        /16773

   len =    1249    nex =        4

    Init   64138   64368          +         0
    Intr   64447   64502          +         0
    Intr   64842   64905          +         0
    Term   65169   65386          +         0

>3702724        /95513

   len =    1636    nex =        3

    Term   74797   74565          -         0
    Intr   75178   74949          -         0
    Init   75971   75545          -         0

>3702724        /6984
```

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Reference No. 2750-942P

```
 len =     3134    nex =        8

   Init    8892     9148         +         0
   Intr    9352     9461         +         0
   Intr    9555     9825         +         0
   Intr    9920    10055         +         0
   Intr   10237    10603         +         0
   Intr   10691    11059         +         0
   Intr   11151    11229         +         0
   Term   11334    11533         +         0

>3702728        /207075

 len =     1647    nex =        8

   Term   10490    10127         -         0
   Intr   10656    10573         -         0
   Intr   10792    10748         -         0
   Intr   10942    10888         -         0
   Intr   11147    11028         -         0
   Intr   11275    11224         -         0
   Intr   11456    11374         -         0
   Init   11773    11604         -         0

>3702728        /5200

 len =      750    nex =        1

   Sngl   25422    24673         -         0

>3702728        /29189

 len =      578    nex =        1

   Sngl   34532    35109         +         0

>3702728        /25501

 len =      939    nex =        2

   Init   39949    40151         +         0
   Term   40345    40887         +         0

>3702729        /7579

 len =     1270    nex =        1

   Sngl   28996    29292         +         0

>3702730        /18256

 len =      614    nex =        1

   Sngl   24396    23783         -         0

>3702731        /37901

 len =     1750    nex =        2

   Init   15709    16151         +         0
```

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```
    Term   17205   17451        +        0

>3702731        /142174

    len =     281   nex =       1

    Sngl   15712   15992        +        0

>3702731        /205672

    len =     617   nex =       1

    Sngl    1863    1247        -        0

>3702731        /126555

    len =    1884   nex =       2

    Term   22481   22203        -        0
    Init   22709   22575        -        0

>3702731        /36901

    len =    2209   nex =       8

    Term    4259    3981        -        0
    Intr    4544    4368        -        0
    Intr    4802    4628        -        0
    Intr    4997    4887        -        0
    Intr    5223    5148        -        0
    Intr    5410    5337        -        0
    Intr    5696    5493        -        0
    Init    6189    6014        -        0

>3702731        /36963

    len =    2086   nex =       6

    Init    7741    8031        +        0
    Intr    8293    8355        +        0
    Intr    8442    8558        +        0
    Intr    8650    9091        +        0
    Intr    9265    9416        +        0
    Term    9532    9826        +        0

>3702731        /27099

    len =    2791   nex =       7

    Init   81625   81712        +        0
    Intr   82523   82571        +        0
    Intr   82720   82828        +        0
    Intr   83013   83106        +        0
    Intr   83540   83673        +        0
    Intr   83776   83939        +        0
    Term   84172   84415        +        0

>3702732        /98232

    len =    2066   nex =       2
```

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```
     Init    13407    13675          +        0
     Term    14812    15472          +        0

>3702732        /19319

   len =     1177    nex =         2

     Init    18687    18864          +        0
     Term    19260    19863          +        0

>3702732        /7700

   len =     1035    nex =         2

     Init    30814    31011          +        0
     Term    31131    31848          +        0

>3702732        /24349

   len =      763    nex =         1

     Sngl    59677    60439          +        0

>3702732        /23018

   len =     2089    nex =         8

     Term    60763    60548          -        0
     Intr    60927    60880          -        0
     Intr    61084    61010          -        0
     Intr    61257    61192          -        0
     Intr    61475    61386          -        0
     Intr    61799    61590          -        0
     Intr    61920    61887          -        0
     Init    62226    62013          -        0

>3702732        /41129

   len =      474    nex =         1

     Sngl     6414     5941          -        0

>3702733        /6599

   len =     2383    nex =         9

     Init    11798    12008          +        0
     Intr    12085    12315          +        0
     Intr    12405    12484          +        0
     Intr    12606    12773          +        0
     Intr    12850    13025          +        0
     Intr    13106    13175          +        0
     Intr    13283    13437          +        0
     Intr    13530    13613          +        0
     Term    13738    14180          +        0

>3702733        /35560

   len =     1653    nex =         4
```

```
     Init   24950   25031        +       0
     Intr   25138   25259        +       0
     Intr   25830   25907        +       0
     Term   25996   26115        +       0

  >3702733        /789

    len =     1531    nex =        1

     Sngl     4266    2736        -       0

  >3702734        /19116

    len =     2455    nex =        9

     Term     9390    9018        -       0
     Intr     9751    9471        -       0
     Intr     9915    9844        -       0
     Intr    10035   10000        -       0
     Intr    10280   10119        -       0
     Intr    10491   10387        -       0
     Intr    10697   10611        -       0
     Intr    11111   10989        -       0
     Init    11472   11255        -       0

  >3702734        /7232

    len =      146    nex =        1

     Sngl     3094    3239        +       0

  >3702734        /121024

    len =     1737    nex =        4

     Init    42608   42742        +       0
     Intr    43086   43196        +       0
     Intr    43298   43408        +       0
     Term    43484   43846        +       0

  >3702734        /10658

    len =      579    nex =        1

     Sngl     4938    5516        +       0

  >3702734        /14354

    len =     1181    nex =        2

     Init      662    1137        +       0
     Term     1645    1842        +       0

  >3702734        /39557

    len =      790    nex =        1

     Sngl     7453    8241        +       0
```

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```
>3702735        /14236

    len =    2126   nex =       7

     Term    9857    9648        -         0
     Intr   10069   10001        -         0
     Intr   10631   10500        -         0
     Intr   10849   10734        -         0
     Intr   11016   10935        -         0
     Intr   11194   11124        -         0
     Init   11773   11547        -         0

>3702735        /29871

    len =    2630   nex =       7

     Term    9857    9489        -         0
     Intr   10069   10001        -         0
     Intr   10631   10500        -         0
     Intr   10849   10734        -         0
     Intr   11016   10935        -         0
     Intr   11194   11124        -         0
     Init   12118   11803        -         0

>3702735        /30034

    len =    1860   nex =       5

     Term   12882   12590        -         0
     Intr   13611   13478        -         0
     Intr   13786   13700        -         0
     Intr   14287   13958        -         0
     Init   14449   14377        -         0

>3702735        /1492

    len =    2013   nex =       7

     Init   15808   15880        +         0
     Intr   16103   16150        +         0
     Intr   16235   16282        +         0
     Intr   16379   16450        +         0
     Intr   16538   16693        +         0
     Intr   16788   16891        +         0
     Term   16967   17259        +         0

>3702735        /39954

    len =    2145   nex =       7

     Term   17743   17402        -         0
     Intr   17875   17828        -         0
     Intr   18119   17961        -         0
     Intr   18361   18220        -         0
     Intr   18509   18444        -         0
     Intr   18923   18605        -         0
     Init   19546   19195        -         0

>3702735        /7451
```

```
      len =      288    nex =        1

        Sngl   19551   19264         -        0

>3702735        /19104

      len =     2153    nex =        7

        Term   17743   17402         -        0
        Intr   17875   17828         -        0
        Intr   18119   17961         -        0
        Intr   18361   18220         -        0
        Intr   18509   18444         -        0
        Intr   18923   18605         -        0
        Init   19554   19195         -        0

>3702735        /37772

      len =     2186    nex =        7

        Term   17743   17415         -        0
        Intr   17875   17828         -        0
        Intr   18119   17961         -        0
        Intr   18361   18220         -        0
        Intr   18509   18444         -        0
        Intr   18923   18605         -        0
        Init   19600   19195         -        0

>3702735        /108736

      len =     2183    nex =        7

        Term   17743   17418         -        0
        Intr   17875   17828         -        0
        Intr   18119   17961         -        0
        Intr   18361   18220         -        0
        Intr   18509   18444         -        0
        Intr   18923   18605         -        0
        Init   19600   19195         -        0

>3702735        /3012

      len =      970    nex =        1

        Sngl   21165   20204         -        0

>3702735        /16025

      len =     1114    nex =        1

        Sngl   28361   29474         +        0

>3702735        /40827

      len =     1870    nex =        7

        Init   32059   32271         +        0
        Intr   32368   32648         +        0
        Intr   32727   32792         +        0
        Intr   32884   33024         +        0
```

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```
        Intr    33122    33250        +        0
        Intr    33351    33494        +        0
        Term    33582    33926        +        0

>3702735        /38864

     len =     2110    nex =        3

        Init    50260    50547        +        0
        Intr    50645    50722        +        0
        Term    50817    51231        +        0

>3702735        /1037

     len =     1950    nex =        4

        Init    52134    52251        +        0
        Intr    52369    52437        +        0
        Intr    52728    52834        +        0
        Term    52940    53499        +        0

>3702735        /22308

     len =      408    nex =        1

        Sngl    53268    53675        +        0

>3702735        /13359

     len =     1750    nex =        7

        Init    61031    61277        +        0
        Intr    61431    61563        +        0
        Intr    61649    61765        +        0
        Intr    61882    61947        +        0
        Intr    62033    62071        +        0
        Intr    62165    62287        +        0
        Term    62370    62773        +        0

>3702735        /26013

     len =     1510    nex =        4

        Term    65133    64653        -        0
        Intr    65309    65230        -        0
        Intr    65703    65393        -        0
        Init    66162    65839        -        0

>3702736        /21033

     len =      685    nex =        1

        Sngl    14295    13611        -        0

>3702736        /22417

     len =     2244    nex =        9

        Init    22337    22446        +        0
        Intr    22746    22848        +        0
```

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```
      Intr   22935   23021        +        0
      Intr   23099   23226        +        0
      Intr   23309   23405        +        0
      Intr   23513   23590        +        0
      Intr   23673   23898        +        0
      Intr   23978   24201        +        0
      Term   24299   24580        +        0

>3702736       /6639

     len =     1465   nex =       2

      Init     2970    3755        +        0
      Term     3857    4434        +        0

>3702736       /150178

     len =      495   nex =       1

      Sngl   41469   41963        +        0

>3702736       /99738

     len =      551   nex =       1

      Sngl   48180   48730        +        0

>3702737       /35235

     len =     2470   nex =      12

      Term   23292   23060        -        0
      Intr   23486   23403        -        0
      Intr   23671   23567        -        0
      Intr   23913   23788        -        0
      Intr   24083   24012        -        0
      Intr   24325   24250        -        0
      Intr   24509   24468        -        0
      Intr   24649   24594        -        0
      Intr   24929   24816        -        0
      Intr   25118   25065        -        0
      Intr   25399   25274        -        0
      Init   25529   25486        -        0

>3702737       /18419

     len =     2968   nex =      13

      Term   23292   23058        -        0
      Intr   23486   23403        -        0
      Intr   23671   23567        -        0
      Intr   23913   23788        -        0
      Intr   24083   24012        -        0
      Intr   24325   24250        -        0
      Intr   24509   24468        -        0
      Intr   24649   24594        -        0
      Intr   24929   24816        -        0
      Intr   25118   25065        -        0
      Intr   25399   25274        -        0
      Intr   25622   25486        -        0
```

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```
     Init   26025   25713         -        0

>3702737       /41468

    len =     1214    nex =       5

     Init   61712   61761         +        0
     Intr   61844   62014         +        0
     Intr   62113   62182         +        0
     Intr   62277   62397         +        0
     Term   62489   62925         +        0

>3702737       /25523

    len =     1270    nex =       2

     Term    9043    8460         -        0
     Init    9725    9132         -        0

>3702738       /3619

    len =     1734    nex =       3

     Init    8288    8505         +        0
     Intr    9426    9614         +        0
     Term    9689   10021         +        0

>3702739       /38027

    len =      342    nex =       1

     Sngl     342       1         -        0

>3702739       /11852

    len =     1734    nex =       1

     Sngl   83965   83716         -        0

>3738088       /29306

    len =     2416    nex =       9

     Term   21390   21051         -        0
     Intr   21586   21492         -        0
     Intr   21773   21684         -        0
     Intr   21894   21859         -        0
     Intr   22056   21973         -        0
     Intr   22222   22133         -        0
     Intr   22628   22314         -        0
     Intr   23079   22864         -        0
     Init   23466   23181         -        0

>3738275       /14182

    len =      975    nex =       2

     Init   18779   18953         +        0
     Term   19387   19753         +        0
```

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```
>3738275       /8386

  len =    1164   nex =      4

   Term   22568   22293      -        0
   Intr   22774   22653      -        0
   Intr   23106   23012      -        0
   Init   23456   23209      -        0

>3738275       /39367

  len =    1990   nex =      8

   Term   38894   38400      -        0
   Intr   39077   38993      -        0
   Intr   39243   39152      -        0
   Intr   39444   39334      -        0
   Intr   39608   39534      -        0
   Intr   39848   39753      -        0
   Intr   40152   39939      -        0
   Init   40384   40234      -        0

>3738275       /21006

  len =     619   nex =      1

   Sngl   72476   73094      +        0

>3738275       /39915

  len =    1487   nex =      4

   Init   75590   76151      +        0
   Intr   76230   76391      +        0
   Intr   76475   76746      +        0
   Term   76819   77076      +        0

>3738275       /10341

  len =     929   nex =      2

   Init   82166   82372      +        0
   Term   82451   83094      +        0

>3738275       /5414

  len =     699   nex =      1

   Sngl   85117   84419      -        0

>3738275       /31153

  len =     587   nex =      1

   Sngl   91661   92247      +        0

>3738275       /7887

  len =    1700   nex =      2
```

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```
    Init   96923   97953       +       0
    Term   98161   98622       +       0

>3738313       /11227

   len =    1048   nex =      1

    Sngl   31224   30177       -       0

>3738313       /14133

   len =    2123   nex =      6

    Init   40926   41117       +       0
    Intr   41351   41508       +       0
    Intr   41614   41805       +       0
    Intr   42263   42416       +       0
    Intr   42517   42709       +       0
    Term   42788   43048       +       0

>3738313       /4850

   len =     976   nex =      4

    Init   45374   45479       +       0
    Intr   45584   45606       +       0
    Intr   45853   45974       +       0
    Term   46056   46332       +       0

>3738313       /13580

   len =    1060   nex =      5

    Init   47214   47374       +       0
    Intr   47473   47666       +       0
    Intr   47779   47863       +       0
    Intr   47946   48011       +       0
    Term   48097   48273       +       0

>3738313       /28044

   len =    3250   nex =     11

    Term   50661   50155       -       0
    Intr   51114   50735       -       0
    Intr   51543   51199       -       0
    Intr   51756   51628       -       0
    Intr   51950   51850       -       0
    Intr   52089   52030       -       0
    Intr   52236   52165       -       0
    Intr   52471   52328       -       0
    Intr   52634   52563       -       0
    Intr   52848   52716       -       0
    Init   53400   52959       -       0

>3738313       /11327

   len =    2590   nex =      6

    Term   65078   64374       -       0
```

```
      Intr   65397   65161         -       0
      Intr   65607   65496         -       0
      Intr   66003   65690         -       0
      Intr   66278   66114         -       0
      Init   66956   66369         -       0

  >3738313       /41558

     len =    2170   nex =        9

      Init   92485   92831         +       0
      Intr   92913   92952         +       0
      Intr   93039   93098         +       0
      Intr   93463   93515         +       0
      Intr   93603   93652         +       0
      Intr   93744   93777         +       0
      Intr   93875   93947         +       0
      Intr   94031   94123         +       0
      Term   94234   94650         +       0

  >3746057       -/21261

     len =    1285   nex =        1

      Sngl   15904   14620         -       0

  >3746057       /36533

     len =    3692   nex =        5

      Init   27102   27418         +       0
      Intr   27863   28034         +       0
      Intr   29868   30043         +       0
      Intr   30142   30300         +       0
      Term   30381   30793         +       0

  >3746057       /10986

     len =     574   nex =        2

      Term   32722   32483         -       0
      Init   33056   32809         -       0

  >3757512       /34622

     len =     922   nex =        3

      Term   11574   11216         -       0
      Intr   11801   11665         -       0
      Init   12137   11918         -       0

  >3757512       /156725

     len =     790   nex =        2

      Term   13186   13046         -       0
      Init   13828   13761         -       0

  >3757512       /36208
```

```
    len =     2715    nex =        5

     Term   11574    11127        -          0
     Intr   11801    11665        -          0
     Intr   12158    11918        -          0
     Intr   12416    12250        -          0
     Init   13186    13044        -          0

>3757512         /17067

    len =     1177    nex =        4

     Init   48329    48418        +          0
     Intr   48697    48796        +          0
     Intr   48877    49001        +          0
     Term   49084    49505        +          0

>3757512         /1629

    len =     1161    nex =        3

     Init   48697    48796        +          0
     Intr   48877    49001        +          0
     Term   49084    49491        +          0

>3757512         /34897

    len =     1056    nex =        3

     Init   48697    48796        +          0
     Intr   48877    49001        +          0
     Term   49084    49445        +          0

>3757512         /103391

    len =      807    nex =        3

     Init   48697    48796        +          0
     Intr   48877    49001        +          0
     Term   49084    49503        +          0

>3757512         /10206

    len =      730    nex =        3

     Term   50124    49809        -          0
     Intr   50347    50233        -          0
     Init   50535    50445        -          0

>3757512         /16224

    len =     4768    nex =       12

     Term   50124    49849        -          0
     Intr   50347    50233        -          0
     Intr   50533    50445        -          0
     Intr   51384    51295        -          0
     Intr   51786    51712        -          0
     Intr   51965    51882        -          0
     Intr   52405    52174        -          0
    Intr   52663   52608        -         0
    Intr   52821   52765        -         0
    Intr   52984   52903        -         0
    Intr   54261   53779        -         0
    Init   54612   54457        -         0

>3763915        /39279

   len =    1270   nex =        2

    Term   19272   18719        -         0
    Init   19986   19634        -         0

>3763915        /20820

   len =    2055   nex =        5

    Init   45580   45662        +         0
    Intr   45753   46015        +         0
    Intr   46153   46221        +         0
    Intr   46358   46416        +         0
    Term   46586   46687        +         0

>3763915        /12017

   len =    2775   nex =        7

    Init   50003   50101        +         0
    Intr   50315   50367        +         0
    Intr   50890   50957        +         0
    Intr   51035   51253        +         0
    Intr   51335   51863        +         0
    Intr   52052   52294        +         0
    Term   52385   52777        +         0

>3763915        /34599

   len =    1286   nex =        5

    Init   56203   56557        +         0
    Intr   56631   56904        +         0
    Intr   56983   57115        +         0
    Intr   57204   57290        +         0
    Term   57378   57488        +         0

>3763915        /311

   len =    4030   nex =       13

    Term   60393   60334        -         0
    Intr   60545   60500        -         0
    Intr   60724   60645        -         0
    Intr   60942   60809        -         0
    Intr   61216   61109        -         0
    Intr   61408   61339        -         0
    Intr   61923   61846        -         0
    Intr   62172   62050        -         0
    Intr   62408   62304        -         0
    Intr   63134   63057        -         0
    Intr   63482   63426        -         0
```

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```
    Intr   63977   63861        -         0
    Init   64363   64190        -         0

>3763915        /31507

   len =    1402   nex =       2

    Init    7990    8335        +         0
    Term    9245    9391        +         0

>3763915        /18447

   len =    1714   nex =       3

    Term   87259   87159        -         0
    Intr   87414   87348        -         0
    Init   87961   87899        -         0

>3763944        /8742

   len =    1230   nex =       4

    Init   16958   17118        +         0
    Intr   17220   17330        +         0
    Intr   17589   17767        +         0
    Term   17874   18187        +         0

>3763944        /36974

   len =    1990   nex =       5

    Init    1865    2006        +         0
    Intr    2096    2209        +         0
    Intr    2304    2531        +         0
    Intr    2952    3391        +         0
    Term    3484    3851        +         0

>3763944        /8686

   len =    1578   nex =       6

    Init   19154   19555        +         0
    Intr   19635   19679        +         0
    Intr   19765   19837        +         0
    Intr   19939   20036        +         0
    Intr   20136   20206        +         0
    Term   20399   20731        +         0

>3763944        /19199

   len =    1228   nex =       6

    Init   19315   19555        +         0
    Intr   19635   19679        +         0
    Intr   19765   19837        +         0
    Intr   19939   20036        +         0
    Intr   20136   20206        +         0
    Term   20399   20542        +         0

>3763944        /9640
```

```
        len =      772   nex =        2

         Init    3064     3391         +         0
         Term    3484     3835         +         0

    >3763944          /40008

        len =     2252   nex =        7

         Term   33666    33350         -         0
         Intr   34075    33766         -         0
         Intr   34206    34161         -         0
         Intr   34967    34896         -         0
         Intr   35185    35059         -         0
         Intr   35397    35293         -         0
         Init   35601    35504         -         0

    >3763944          /3321

        len =     1486   nex =        6

         Term   48162    48090         -         0
         Intr   48339    48287         -         0
         Intr   48651    48603         -         0
         Intr   48901    48837         -         0
         Intr   49077    48990         -         0
         Init   49575    49178         -         0

    >3763944          /35300

        len =      339   nex =        1

         Sngl   54201    53863         -         0

    >3763944          /37313

        len =     2087   nex =        6

         Init   65398    65640         +         0
         Intr   65729    65831         +         0
         Intr   66198    66283         +         0
         Intr   66459    66697         +         0
         Intr   66803    66992         +         0
         Term   67215    67484         +         0

    >3763944          /16451

        len =     1295   nex =        3

         Term   78903    78431         -         0
         Intr   79217    79086         -         0
         Init   79720    79611         -         0

    >3763944          /8049

        len =     1390   nex =        3

         Term   78903    78426         -         0
         Intr   79217    79086         -         0
```

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```
     Init   79807   79611          -         0

  >3763944        /43034

    len =     386    nex =        1

     Sngl   79807   79611          -         0

  >3766106        /122624

    len =     190    nex =        1

     Sngl   13182   12996          -         0

  >3766106        /17815

    len =    4706    nex =       19

     Term   13428   12997          -         0
     Intr   13611   13523          -         0
     Intr   13786   13708          -         0
     Intr   13982   13885          -         0
     Intr   14288   14184          -         0
     Intr   14458   14372          -         0
     Intr   14840   14646          -         0
     Intr   15050   14944          -         0
     Intr   15272   15140          -         0
     Intr   15413   15351          -         0
     Intr   15600   15499          -         0
     Intr   15756   15685          -         0
     Intr   15905   15849          -         0
     Intr   16194   16093          -         0
     Intr   16361   16324          -         0
     Intr   16560   16449          -         0
     Intr   16723   16667          -         0
     Intr   16859   16804          -         0
     Init   17096   16970          -         0

  >3766106        /31830

    len =    1468    nex =        1

     Sngl   21312   20870          -         0

  >3766106        /2763

    len =    1609    nex =        7

     Term   25723   25696          -         0
     Intr   25940   25843          -         0
     Intr   26117   26044          -         0
     Intr   26241   26200          -         0
     Intr   26573   26515          -         0
     Intr   26960   26874          -         0
     Init   27166   27008          -         0

  >3766106        /19071

    len =    1931    nex =        8
```

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```
    Term   25723   25503          -         0
    Intr   25940   25843          -         0
    Intr   26117   26044          -         0
    Intr   26241   26200          -         0
    Intr   26573   26515          -         0
    Intr   26960   26874          -         0
    Intr   27166   27139          -         0
    Init   27433   27265          -         0

>3766106        /772

   len =     1100    nex =        3

    Init   55679   55811          +         0
    Intr   55892   56190          +         0
    Term   56287   56543          +         0

>3766106        /42210

   len =     1404    nex =        4

    Term    4966    4948          -         0
    Intr    5280    5077          -         0
    Intr    5648    5451          -         0
    Init    6351    6198          -         0

>3766106        /37467

   len =     3610    nex =       15

    Init    6698    6918          +         0
    Intr    7000    7028          +         0
    Intr    7114    7151          +         0
    Intr    7313    7400          +         0
    Intr    7670    7775          +         0
    Intr    7890    7960          +         0
    Intr    8194    8263          +         0
    Intr    8365    8427          +         0
    Intr    8718    8789          +         0
    Intr    8898    8999          +         0
    Intr    9219    9293          +         0
    Intr    9415    9513          +         0
    Intr    9747    9863          +         0
    Intr    9947   10003          +         0
    Term   10101   10306          +         0

>3766106        /31765

   len =     1407    nex =        5

    Term   82104   82012          -         0
    Intr   82327   82239          -         0
    Intr   82516   82454          -         0
    Intr   82689   82600          -         0
    Init   82977   82917          -         0

>3766106        /32117

   len =     2329    nex =        9
```

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```
     Term   81812   81701       -        0
     Intr   82104   82012       -        0
     Intr   82327   82239       -        0
     Intr   82516   82454       -        0
     Intr   82689   82600       -        0
     Intr   82977   82917       -        0
     Intr   83164   83070       -        0
     Intr   83370   83281       -        0
     Init   84029   83633       -        0

 >3779020       /7600

    len =     928    nex =       3

     Term   43560   43284       -        0
     Intr   43921   43879       -        0
     Init   44211   44021       -        0

 >3785968       /41002

    len =    2151    nex =       9

     Init   47820   47930       +        0
     Intr   48026   48147       +        0
     Intr   48227   48287       +        0
     Intr   48637   48705       +        0
     Intr   48785   48903       +        0
     Intr   49026   49173       +        0
     Intr   49271   49415       +        0
     Intr   49514   49600       +        0
     Term   49702   49962       +        0

 >3785968       /18820

    len =    2037    nex =       4

     Term   51353   50716       -        0
     Intr   51853   51786       -        0
     Intr   52406   52062       -        0
     Init   52752   52479       -        0

 >3785968       /11949

    len =    1210    nex =       1

     Sngl   56312   55108       -        0

 >3785968       /35997

    len =    1090    nex =       2

     Init   62489   62738       +        0
     Term   63173   63578       +        0

 >3785968       /17603

    len =    1210    nex =       2

     Init   62489   62738       +        0
     Term   63173   63691       +        0
```

```
>3785968        /2422

    len =     956   nex =       2

     Init     832     994         +        0
     Term    1320    1787         +        0

>3785968        /32861

    len =    1930   nex =       5

     Init   97394   97474         +        0
     Intr   97567   97743         +        0
     Intr   97843   97921         +        0
     Intr   98036   98151         +        0
     Term   98536   99144         +        0

>3785992        /3294

    len =    1600   nex =       6

     Term   14853   14529         -        0
     Intr   15039   14947         -        0
     Intr   15343   15131         -        0
     Intr   15667   15434         -        0
     Intr   15888   15743         -        0
     Init   16128   15979         -        0

>3785992        /32721

    len =    2131   nex =       6

     Init   18649   18757         +        0
     Intr   18865   19046         +        0
     Intr   19570   19788         +        0
     Intr   19870   20091         +        0
     Intr   20183   20254         +        0
     Term   20343   20779         +        0

>3785992        /40283

    len =    1785   nex =       4

     Init   21029   21179         +        0
     Intr   21550   21664         +        0
     Intr   21810   21961         +        0
     Term   22069   22813         +        0

>3785992        /17861

    len =    2802   nex =       5

     Init   30317   30479         +        0
     Intr   30906   31025         +        0
     Intr   31867   31981         +        0
     Intr   32237   32330         +        0
     Term   32412   32768         +        0

>3785992        /35493
```

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```
     len =     3170    nex =        9

      Init    33260    33564           +          0
      Intr    33721    33919           +          0
      Intr    34406    34469           +          0
      Intr    34703    34847           +          0
      Intr    35156    35242           +          0
      Intr    35398    35555           +          0
      Intr    35655    35703           +          0
      Intr    35816    35940           +          0
      Term    36021    36429           +          0

  >3785992        /37377

     len =     2858    nex =       10

      Init    33355    33564           +          0
      Intr    33721    33919           +          0
      Intr    34406    34469           +          0
      Intr    34703    34847           +          0
      Intr    35156    35242           +          0
      Intr    35398    35555           +          0
      Intr    35655    35703           +          0
      Intr    35816    35940           +          0
      Intr    36021    36123           +          0
      Term    36144    36212           +          0

  >3785992        /21746

     len =     2965    nex =        9

      Term    45531    45288           -          0
      Intr    45743    45645           -          0
      Intr    45891    45826           -          0
      Intr    46097    45977           -          0
      Intr    46418    46351           -          0
      Intr    46701    46630           -          0
      Intr    47414    47230           -          0
      Intr    47749    47514           -          0
      Init    48252    47839           -          0

  >3785992        /101298

     len =      250    nex =        1

      Sngl    82983    82739           -          0

  >3785992        /25626

     len =     2398    nex =        6

      Term    83028    82729           -          0
      Intr    83330    83293           -          0
      Intr    83469    83420           -          0
      Intr    83997    83830           -          0
      Intr    84473    84083           -          0
      Init    85126    84720           -          0

  >3785992        /18328
```

```
      len =      730    nex =        2

       Term   88201    87996         -         0
       Init   88716    88313         -         0

  >3789706         /18060

      len =     1375    nex =        2

       Init   17339    17476         +         0
       Term   18116    18387         +         0

  >3789706         /40461

      len =     1943    nex =        7

       Init   41058    41294         +         0
       Intr   41427    41497         +         0
       Intr   41746    41854         +         0
       Intr   41986    42068         +         0
       Intr   42216    42374         +         0
       Intr   42466    42567         +         0
       Term   42665    43000         +         0

  >3789706         /12454

      len =     1482    nex =        1

       Sngl   49272    47791         -         0

  >3789706         /36143

      len =     1053    nex =        1

       Sngl   66764    65712         -         0

  >3789706         /112437

      len =      695    nex =        0

  >3805839         /32754

      len =     2297    nex =        8

       Term   16983    16533         -         0
       Intr   17156    17087         -         0
       Intr   17682    17566         -         0
       Intr   17899    17800         -         0
       Intr   18015    17992         -         0
       Intr   18268    18128         -         0
       Intr   18613    18591         -         0
       Init   18829    18688         -         0

  >3805839         /119300

      len =     1606    nex =        5

       Init   44348    44802         +         0
       Intr   44996    45173         +         0
```

```
        Intr    45268    45351          +          0
        Intr    45442    45609          +          0
        Term    45706    45953          +          0

>3805839        /5482

      len =     3370    nex =       12

        Init    47368    47506          +          0
        Intr    47679    47841          +          0
        Intr    48042    48107          +          0
        Intr    48209    48294          +          0
        Intr    48479    48596          +          0
        Intr    48680    48886          +          0
        Intr    49008    49084          +          0
        Intr    49250    49343          +          0
        Intr    49606    49671          +          0
        Intr    49774    49851          +          0
        Intr    49955    50056          +          0
        Term    50145    50455          +          0

>3805839        /17725

      len =     2056    nex =        6

        Term    50772    50483          -          0
        Intr    50949    50881          -          0
        Intr    51247    51043          -          0
        Intr    51692    51610          -          0
        Intr    52241    52102          -          0
        Init    52538    52356          -          0

>3805839        /1095

      len =     1127    nex =        3

        Term    70923    70590          -          0
        Intr    71436    71382          -          0
        Init    71716    71529          -          0

>3805839        /8360

      len =     1095    nex =        3

        Term    94509    94155          -          0
        Intr    94708    94595          -          0
        Init    95249    95131          -          0

>3831437        /3743

      len =      941    nex =        3

        Init     6216     6447          +          0
        Intr     6552     6618          +          0
        Term     6704     7156          +          0

>3831448        /9671

      len =     1169    nex =        2
```

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```
          Init    40447    40831         +        0
          Term    41264    41615         +        0

        >3831448        /17467

         len =      395    nex =        1

          Sngl    61543    61937         +        0

        >3831448        /35104

         len =     1522    nex =        1

          Sngl     8526     7008         -        0

        >3831448        /37237

         len =     3011    nex =        7

          Term    88224    87712         -        0
          Intr    88432    88334         -        0
          Intr    88649    88483         -        0
          Intr    89505    89463         -        0
          Intr    89768    89673         -        0
          Intr    89984    89868         -        0
          Init    90722    90415         -        0

        >3849811        /9623

         len =     1165    nex =        4

          Term      896      533         -        0
          Intr     1195      997         -        0
          Intr     1462     1307         -        0
          Init     1697     1546         -        0

        >3849811        /1568

         len =     1042    nex =        4

          Term    23646    23559         -        0
          Intr    23842    23733         -        0
          Intr    24392    24313         -        0
          Init    24600    24516         -        0

        >3849811        /30470

         len =     1123    nex =        3

          Term    23842    23486         -        0
          Intr    24392    24313         -        0
          Init    24608    24516         -        0

        >3849811        /7182

         len =     1193    nex =        4

          Term    23646    23450         -        0
          Intr    23842    23733         -        0
          Intr    24392    24313         -        0
```

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```
     Init   24642   24516        -        0

>3849811        /36468

    len =     1339   nex =        1

     Sngl   28830   27492        -        0

>3849811        /33391

    len =     1311   nex =        3

     Init   29563   29631        +        0
     Intr   29985   30486        +        0
     Term   30573   30873        +        0

>3849811        /35307

    len =      730   nex =        2

     Init   29985   30486        +        0
     Term   30573   30713        +        0

>3849811        /24946

    len =      772   nex =        1

     Sngl   37425   38196        +        0

>3849811        /19020

    len =     2018   nex =        6

     Init   51039   51216        +        0
     Intr   51447   51560        +        0
     Intr   51656   51804        +        0
     Intr   51969   52041        +        0
     Intr   52302   52341        +        0
     Term   52685   52767        +        0

>3849811        /18200

    len =     1310   nex =        2

     Init   59367   59612        +        0
     Term   60232   60674        +        0

>3849811        /12305

    len =      512   nex =        1

     Sngl    5941    6452        +        0

>3859590        /34747

    len =      741   nex =        3

     Term   39398   39023        -        0
     Intr   39602   39499        -        0
     Init   39763   39682        -        0
```

```
>3859590        /36898

    len =     1476    nex =        2

     Init    53829    54125         +        0
     Term    54213    54342         +        0

>3859590        /15259

    len =      343    nex =        1

     Sngl    58875    59217         +        0

>3859590        /20761

    len =     2429    nex =       11

     Term    74605    74285         -        0
     Intr    74729    74694         -        0
     Intr    74938    74825         -        0
     Intr    75236    75018         -        0
     Intr    75451    75326         -        0
     Intr    75613    75539         -        0
     Intr    75707    75703         -        0
     Intr    75970    75814         -        0
     Intr    76141    76061         -        0
     Intr    76278    76225         -        0
     Init    76713    76474         -        0

>3859590        /95621

    len =     1150    nex =        4

     Term    77280    77093         -        0
     Intr    77795    77518         -        0
     Intr    77994    77893         -        0
     Init    78208    78069         -        0

>3859590        /15383

    len =     1038    nex =        3

     Term    84801    84590         -        0
     Intr    85252    84874         -        0
     Init    85627    85486         -        0

>3859590        /29072

    len =      712    nex =        1

     Sngl    88825    89536         +        0

>3859658        /17194

    len =      515    nex =        1

     Sngl    16172    16686         +        0

>3859658        /107993
```

```
   len =      586    nex =        1

    Sngl   16174   16759          +        0

>3859658         /33603

   len =      409    nex =        1

    Sngl   16552   16144          -        0

>3859658         /6589

   len =     2209    nex =        9

    Init   20101   20257          +        0
    Intr   20354   20412          +        0
    Intr   20723   20875          +        0
    Intr   20969   21017          +        0
    Intr   21116   21241          +        0
    Intr   21326   21379          +        0
    Intr   21582   21664          +        0
    Intr   21751   21856          +        0
    Term   21928   22293          +        0

>3859658         /29040

   len =      744    nex =        3

    Init   21625   21664          +        0
    Intr   21751   21856          +        0
    Term   21928   22368          +        0

>3859658         /31672

   len =      712    nex =        2

    Term   40705   40329          -        0
    Init   41020   40903          -        0

>3859658         /109432

   len =      610    nex =        2

    Term   48314   48122          -        0
    Init   48723   48437          -        0

>3859658         /38416

   len =      403    nex =        1

    Sngl   49905   50307          +        0

>3859658         /29886

   len =     2037    nex =        5

    Term   50573   50297          -        0
    Intr   50791   50663          -        0
    Intr   51573   51274          -        0
```

```
     Intr   51886   51658          -        0
     Init   52333   52049          -        0

  >3859658        /38404

    len =    2978   nex =       10

     Term   52912   52479          -        0
     Intr   53209   53003          -        0
     Intr   53605   53285          -        0
     Intr   53757   53707          -        0
     Intr   53947   53843          -        0
     Intr   54222   54160          -        0
     Intr   54623   54507          -        0
     Intr   54886   54819          -        0
     Intr   55081   55005          -        0
     Init   55456   55320          -        0

  >3859658        /5743

    len =     790   nex =        1

     Sngl   63524   64304          +        0

  >3859658        /6636

    len =    3730   nex =        9

     Term   85246   84985          -        0
     Intr   85487   85360          -        0
     Intr   85826   85560          -        0
     Intr   85995   85914          -        0
     Intr   86282   86121          -        0
     Intr   86558   86366          -        0
     Intr   87259   87159          -        0
     Intr   87578   87376          -        0
     Init   88293   88190          -        0

  >3859658        /6375

    len =    1398   nex =        3

     Term   91234   91166          -        0
     Intr   91430   91335          -        0
     Init   92183   91722          -        0

  >3860242        /1860

    len =     833   nex =        2

     Init   19578   19983          +        0
     Term   20109   20390          +        0

  >3860242        /123227

    len =    1390   nex =        4

     Init   22933   23272          +        0
     Intr   23512   23607          +        0
     Intr   23806   23845          +        0
```

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```
    Term   24006   24320         +         0

>3860242        /29893

    len =     1317    nex =        4

    Init   38909   39035         +         0
    Intr   39156   39214         +         0
    Intr   39796   39839         +         0
    Term   39982   40225         +         0

>3860242        /40339

    len =      705    nex =        1

    Sngl   45860   45156         -         0

>3860242        /38650

    len =     1396    nex =        1

    Sngl   46542   45147         -         0

>3860242        /25214

    len =     2937    nex =        4

    Term   50568   50049         -         0
    Intr   50808   50653         -         0
    Intr   51048   50902         -         0
    Init   52985   52151         -         0

>3860242        /21711

    len =     2316    nex =        9

    Init   62073   62233         +         0
    Intr   62317   62379         +         0
    Intr   62477   62543         +         0
    Intr   62938   62988         +         0
    Intr   63081   63161         +         0
    Intr   63509   63621         +         0
    Intr   63713   63830         +         0
    Intr   63931   64057         +         0
    Term   64162   64388         +         0

>3860242        /96159

    len =     1407    nex =        4

    Term   64781   64588         -         0
    Intr   64990   64893         -         0
    Intr   65621   65504         -         0
    Init   65994   65896         -         0

>3860243        /14203

    len =      949    nex =        0

>3860243        /19048
```

```
      len =     1570    nex =        4

       Init  115309   115528         +          0
       Intr  115614   115764         +          0
       Intr  115886   116091         +          0
       Term  116181   116399         +          0

   >3860243        /28205

      len =     2483    nex =        9

       Init    12779    13109        +          0
       Intr    13268    13360        +          0
       Intr    13462    13569        +          0
       Intr    13849    13963        +          0
       Intr    14039    14172        +          0
       Intr    14261    14362        +          0
       Intr    14472    14564        +          0
       Intr    14654    14789        +          0
       Term    14881    15261        +          0

   >3860243        /6892

      len =     2155    nex =        8

       Init    12829    13109        +          0
       Intr    13268    13360        +          0
       Intr    13462    13569        +          0
       Intr    13849    13963        +          0
       Intr    14039    14172        +          0
       Intr    14261    14362        +          0
       Intr    14472    14564        +          0
       Term    14654    14983        +          0

   >3860243        /113089

      len =     1489    nex =        5

       Init    13495    13963        +          0
       Intr    14039    14172        +          0
       Intr    14261    14362        +          0
       Intr    14472    14564        +          0
       Term    14654    14983        +          0

   >3860243        /23972

      len =     1889    nex =        7

       Term    16019    15795        -          0
       Intr    16256    16115        -          0
       Intr    16406    16348        -          0
       Intr    16535    16500        -          0
       Intr    16972    16620        -          0
       Intr    17135    17065        -          0
       Init    17683    17580        -          0

   >3860243        /33700

      len =      910    nex =        1
```

```
    Sngl   19112   18209          -        0

>3860243        /37739

  len =     1518   nex =        1

    Sngl   22909   21392          -        0

>3860243        /112146

  len =     1001   nex =        2

    Init   36410   36592          +        0
    Term   36690   36790          +        0

>3860243        /3359

  len =     2034   nex =        3

    Init   58793   58908          +        0
    Intr   59274   59312          +        0
    Term   59418   59832          +        0

>3860243        /19986

  len =     1037   nex =        4

    Term   60333   60150          -        0
    Intr   60655   60423          -        0
    Intr   60984   60894          -        0
    Init   61186   61074          -        0

>3860243        /207594

  len =      407   nex =        2

    Term   62033   61867          -        0
    Init   62273   62139          -        0

>3860243        /9845

  len =     1062   nex =        3

    Term   62033   61843          -        0
    Intr   62371   62139          -        0
    Init   62751   62661          -        0

>3860243        /120911

  len =      959   nex =        3

    Term   62033   61946          -        0
    Intr   62371   62139          -        0
    Init   62751   62661          -        0

>3860243        /36076

  len =     1039   nex =        3
```

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```
      Term    62033    61867         -          0
      Intr    62371    62139         -          0
      Init    62751    62661         -          0

   >3860243        /6418

      len =     2090    nex =        9

      Init    73691    74012         +          0
      Intr    74216    74300         +          0
      Intr    74385    74501         +          0
      Intr    74585    74649         +          0
      Intr    74744    74832         +          0
      Intr    74933    75012         +          0
      Intr    75094    75205         +          0
      Intr    75307    75426         +          0
      Term    75511    75780         +          0

   >3860243        /3818

      len =     2891    nex =        8

      Term    96072    95749         -          0
      Intr    96363    96208         -          0
      Intr    96564    96466         -          0
      Intr    96729    96655         -          0
      Intr    96877    96809         -          0
      Intr    97159    96961         -          0
      Intr    97879    97774         -          0
      Init    98639    97974         -          0

   >3868722        /125396

      len =      179    nex =        1

      Sngl    14027    13849         -          0

   >3868723        /35059

      len =     3373    nex =        6

      Init    47360    47692         +          0
      Intr    47785    47840         +          0
      Intr    47939    47993         +          0
      Intr    49588    49668         +          0
      Intr    49756    49979         +          0
      Term    50524    50732         +          0

   >3868723        /101605

      len =      415    nex =        1

      Sngl    52231    51821         -          0

   >3868723        /42560

      len =     1118    nex =        2

      Term    62440    61893         -          0
      Init    63010    62468         -          0
```

```
>3869062        /15995

    len =     1300    nex =        2

      Init    5593     5774          +          0
      Term    5873     6151          +          0

>3869063        /12997

    len =     1210    nex =        2

      Init   39503    39620          +          0
      Term   39709    40708          +          0

>3869063        /2393

    len =      813    nex =        1

      Sngl   49706    50518          +          0

>3869063        /37862

    len =     2178    nex =        7

      Init   65062    65329          +          0
      Intr   65646    65723          +          0
      Intr   65820    65987          +          0
      Intr   66119    66167          +          0
      Intr   66275    66348          +          0
      Intr   66564    66657          +          0
      Term   66756    67239          +          0

>3869064        /37349

    len =     2599    nex =        8

      Term    1690      525          -          0
      Intr    1966     1776          -          0
      Intr    2274     2069          -          0
      Intr    2446     2367          -          0
      Intr    2588     2535          -          0
      Intr    2723     2663          -          0
      Intr    2912     2839          -          0
      Init    3118     2994          -          0

>3869065        /119129

    len =     1317    nex =        3

      Term   30028    29747          -          0
      Intr   30375    30116          -          0
      Init   31063    30948          -          0

>3869065        /17872

    len =     1112    nex =        3

      Term   36192    35871          -          0
      Intr   36608    36279          -          0
```

```
     Init   36982   36828        -        0

>3869065        /28066

     len =     202   nex =       1

     Sngl   58498   58699        +        0

>3869065        /29368

     len =    2470   nex =       7

     Init   65929   66029        +        0
     Intr   66279   66586        +        0
     Intr   66669   66764        +        0
     Intr   66861   66938        +        0
     Intr   67036   67212        +        0
     Intr   67884   67952        +        0
     Term   68051   68397        +        0

>3869066        /40790

     len =    2119   nex =       7

     Init   35935   36190        +        0
     Intr   36417   36492        +        0
     Intr   36599   36672        +        0
     Intr   36770   36975        +        0
     Intr   37095   37410        +        0
     Intr   37502   37655        +        0
     Term   37756   38053        +        0

>3869066        /118207

     len =     685   nex =       1

     Sngl   47112   46433        -        0

>3869067        /33343

     len =    2470   nex =       8

     Init   22709   22777        +        0
     Intr   22866   22916        +        0
     Intr   23160   23271        +        0
     Intr   23380   23424        +        0
     Intr   23921   24046        +        0
     Intr   24373   24479        +        0
     Intr   24569   24640        +        0
     Term   24751   25178        +        0

>3869067        /20436

     len =    2137   nex =       5

     Init   25230   25389        +        0
     Intr   25682   25841        +        0
     Intr   25964   26519        +        0
     Intr   26612   26883        +        0
     Term   27050   27366        +        0
```

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```
>3869067        /36010

   len =     2118    nex =        6

     Init   29168    29584         +         0
     Intr   29688    30014         +         0
     Intr   30180    30332         +         0
     Intr   30470    30594         +         0
     Intr   30707    30802         +         0
     Term   30894    31285         +         0

>3869067        /5542

   len =     1210    nex =        5

     Term   31596    31244         -         0
     Intr   31767    31669         -         0
     Intr   31964    31870         -         0
     Intr   32299    32185         -         0
     Init   32449    32382         -         0

>3869067        /12033

   len =     1338    nex =        3

     Init   32650    32895         +         0
     Intr   32975    33207         +         0
     Term   33308    33974         +         0

>3869067        /3488

   len =     1018    nex =        4

     Term   34260    34052         -         0
     Intr   34424    34339         -         0
     Intr   34606    34490         -         0
     Init   35069    34689         -         0

>3869067        /40511

   len =     2721    nex =       11

     Init   37516    37614         +         0
     Intr   37720    37836         +         0
     Intr   37991    38076         +         0
     Intr   38371    38459         +         0
     Intr   38556    38611         +         0
     Intr   38753    38833         +         0
     Intr   39082    39147         +         0
     Intr   39226    39318         +         0
     Intr   39654    39718         +         0
     Intr   39813    39948         +         0
     Term   40028    40236         +         0

>3869067        /41451

   len =      933    nex =        1

     Sngl    4200     3268         -         0
```

```
>3869067          /123228

   len =      999    nex =        3

     Term   48536    48214         -          0
     Intr   48756    48626         -          0
     Init   49212    48805         -          0

>3869067          /25200

   len =     2567    nex =       12

     Term   53194    52902         -          0
     Intr   53329    53276         -          0
     Intr   53480    53412         -          0
     Intr   53617    53549         -          0
     Intr   53823    53701         -          0
     Intr   54011    53910         -          0
     Intr   54167    54093         -          0
     Intr   54334    54244         -          0
     Intr   54762    54425         -          0
     Intr   54939    54853         -          0
     Intr   55148    55058         -          0
     Init   55468    55243         -          0

>3869067          /17375

   len =      576    nex =        2

     Init   69414    69652         +          0
     Term   69732    69989         +          0

>3869068          /17883

   len =      535    nex =        1

     Sngl   16765    16231         -          0

>3869068          /30700

   len =      310    nex =        1

     Sngl   18011    17704         -          0

>3869068          /29310

   len =     2516    nex =        5

     Term   18580    17714         -          0
     Intr   19092    18662         -          0
     Intr   19286    19183         -          0
     Intr   19439    19371         -          0
     Init   20229    20088         -          0

>3869068          /112999

   len =      658    nex =        1

     Sngl   27286    27943         +          0
```

```
>3869068        /10694

   len =     556   nex =        1

     Sngl   82911   82356        -        0

>3869069        /106959

   len =     404   nex =        1

     Sngl    2091    1688        -        0

>3869069        /12707

   len =    3200   nex =        7

     Init   30363   30606        +        0
     Intr   31683   31882        +        0
     Intr   32143   32357        +        0
     Intr   32433   32523        +        0
     Intr   32605   32908        +        0
     Intr   33006   33066        +        0
     Term   33143   33562        +        0

>3869069        /101843

   len =    2862   nex =       10

     Init   33963   34200        +        0
     Intr   34320   34353        +        0
     Intr   34515   34655        +        0
     Intr   34751   34838        +        0
     Intr   34915   34995        +        0
     Intr   35079   35143        +        0
     Intr   35303   35443        +        0
     Intr   35530   35643        +        0
     Intr   35847   35877        +        0
     Term   36333   36824        +        0

>3869069        /15577

   len =    1904   nex =        3

     Term    2612    1677        -        0
     Intr    3118    2808        -        0
     Init    3580    3284        -        0

>3869069        /36656

   len =    1892   nex =        3

     Term    2612    1692        -        0
     Intr    3118    2808        -        0
     Init    3583    3284        -        0

>3869069        /206508

   len =    1588   nex =        2
```

```
     Init   39936   40013         +        0
     Term   40104   40647         +        0

>3869069       /20484

   len =    1176   nex =       5

     Term   52941   52689         -        0
     Intr   53185   53027         -        0
     Intr   53373   53284         -        0
     Intr   53608   53463         -        0
     Init   53864   53704         -        0

>3869069       /1490

   len =    1592   nex =       5

     Term   54883   54300         -        0
     Intr   55072   54965         -        0
     Intr   55295   55169         -        0
     Intr   55587   55538         -        0
     Init   55891   55681         -        0

>3869069       /5507

   len =    2151   nex =       9

     Init    7968    8130         +        0
     Intr    8391    8430         +        0
     Intr    8506    8598         +        0
     Intr    8713    8761         +        0
     Intr    8841    8947         +        0
     Intr    9024    9111         +        0
     Intr    9197    9400         +        0
     Intr    9480    9533         +        0
     Term    9618   10118         +        0

>3869069       /33741

   len =     431   nex =       1

     Sngl    9688   10112         +        0

>3869070       /12673

   len =     610   nex =       1

     Sngl    2159    1550         -        0

>3869071       /486

   len =    2937   nex =       9

     Term   11670   11336         -        0
     Intr   12043   11759         -        0
     Intr   12404   12123         -        0
     Intr   12784   12477         -        0
     Intr   13308   12859         -        0
     Intr   13539   13389         -        0
     Intr   13791   13627         -        0
```

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```
                                          1270
   Intr    13989   13873         -          0
   Init    14272   14165         -          0

 >3869071        /38585

  len =     1270   nex =        5

   Term    16190   16024         -          0
   Intr    16555   16484         -          0
   Intr    16728   16660         -          0
   Intr    16951   16821         -          0
   Init    17290   17177         -          0

 >3869071        /9813

  len =     1278   nex =        6

   Term    16190   16019         -          0
   Intr    16395   16323         -          0
   Intr    16555   16484         -          0
   Intr    16728   16660         -          0
   Intr    16951   16821         -          0
   Init    17296   17177         -          0

 >3869071        /7192

  len =      839   nex =        1

   Sngl    30021   29183         -          0

 >3869071        /22955

  len =      730   nex =        1

   Sngl    38598   37870         -          0

 >3869071        /38211

  len =     2531   nex =        7

   Term    57057   56592         -          0
   Intr    57299   57147         -          0
   Intr    57562   57440         -          0
   Intr    57858   57674         -          0
   Intr    58188   58099         -          0
   Intr    58480   58304         -          0
   Init    58813   58761         -          0

 >3869072        /94805

  len =      862   nex =        3

   Init    18597   18828         +          0
   Intr    18912   19014         +          0
   Term    19090   19458         +          0

 >3869072        /17342

  len =      956   nex =        2
```

```
      Term   31251   30971          -       0
      Init   31926   31676          -       0

  >3869072        /158846

     len =     977    nex =       2

      Term   31251   30955          -       0
      Init   31911   31676          -       0

  >3869072        /117248

     len =    1065    nex =       2

      Term   31251   30869          -       0
      Init   31933   31676          -       0

  >3869072        /8695

     len =    1011    nex =       2

      Term   31251   30923          -       0
      Init   31933   31676          -       0

  >3869072        /20422

     len =     821    nex =       2

      Term   31251   31113          -       0
      Init   31933   31676          -       0

  >3869072        /7107

     len =    2532    nex =       7

      Term   32844   32601          -       0
      Intr   33363   33255          -       0
      Intr   33767   33623          -       0
      Intr   34032   33877          -       0
      Intr   34209   34148          -       0
      Intr   34421   34295          -       0
      Init   34651   34556          -       0

  >3869072        /18208

     len =     861    nex =       1

      Sngl   44310   44409          +       0

  >3869072        /26609

     len =     190    nex =       1

      Sngl   53681   53499          -       0

  >3869072        /39314

     len =    1182    nex =       3

      Term   53837   53492          -       0
```

```
         Intr    54380    54210          -          0
         Init    54673    54468          -          0

      >3869073         /5685

        len =      537    nex =         1

         Sngl    10605    11141          +          0

      >3869073         /36279

        len =      458    nex =         1

         Sngl    18587    19044          +          0

      >3869074         /102795

        len =      632    nex =         1

         Sngl    10415     9784          -          0

      >3869074         /7236

        len =     1613    nex =         7

         Init    40937    41184          +          0
         Intr    41262    41385          +          0
         Intr    41618    41726          +          0
         Intr    41836    41895          +          0
         Intr    41988    42053          +          0
         Intr    42160    42251          +          0
         Term    42351    42549          +          0

      >3869074         /7412

        len =     2576    nex =        11

         Term     2325     1773          -          0
         Intr     2504     2410          -          0
         Intr     2685     2589          -          0
         Intr     2944     2776          -          0
         Intr     3117     3057          -          0
         Intr     3305     3204          -          0
         Intr     3550     3439          -          0
         Intr     3788     3634          -          0
         Intr     3973     3903          -          0
         Intr     4165     4054          -          0
         Init     4344     4288          -          0

      >3869074         /19991

        len =     1171    nex =         4

         Term    42980    42681          -          0
         Intr    43303    43190          -          0
         Intr    43699    43568          -          0
         Init    43851    43806          -          0

      >3869074         /16926
```

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```
     len =     1210    nex =        4

      Term    42980    42679          -         0
      Intr    43303    43190          -         0
      Intr    43699    43568          -         0
      Init    43876    43806          -         0

  >3869074          /8940

     len =     3654    nex =        13

      Term     2325     1767          -         0
      Intr     2504     2410          -         0
      Intr     2685     2589          -         0
      Intr     2944     2776          -         0
      Intr     3117     3057          -         0
      Intr     3305     3204          -         0
      Intr     3550     3439          -         0
      Intr     3788     3634          -         0
      Intr     3973     3903          -         0
      Intr     4165     4054          -         0
      Intr     4428     4288          -         0
      Intr     4653     4519          -         0
      Init     5420     4898          -         0

  >3869074          /149198

     len =      278    nex =        1

      Sngl    58874    59151          +         0

  >3869074          /40315

     len =      414    nex =        1

      Sngl    58874    59287          +         0

  >3869074          /154419

     len =      433    nex =        1

      Sngl    58874    59306          +         0

  >3869074          /29762

     len =      854    nex =        1

      Sngl    58874    59727          +         0

  >3869074          /9669

     len =      790    nex =        1

      Sngl    58941    59724          +         0

  >3869074          /11244

     len =      430    nex =        1

      Sngl    59119    59548          +         0
```

```
>3869074        /2815

    len =      671   nex =        1

     Sngl   62233   62903        +         0

>3869074        /113158

    len =      405   nex =        1

     Sngl   73937   73533        -         0

>3869074        /10401

    len =      491   nex =        1

     Sngl   74021   73531        -         0

>3869074        /24707

    len =      730   nex =        2

     Term   74030   73533        -         0
     Init   74252   74212        -         0

>3869074        /206301

    len =      692   nex =        2

     Term   74030   73784        -         0
     Init   74475   74212        -         0

>3869074        /39446

    len =     1883   nex =        3

     Term   74030   73781        -         0
     Intr   74502   74212        -         0
     Init   75663   74775        -         0

>3869074        /28168

    len =     1097   nex =        2

     Term    8429    7843        -         0
     Init    8939    8715        -         0

>3869075        /97900

    len =      937   nex =        2

     Init   10363   10398        +         0
     Term   10768   11299        +         0

>3869075        /13188

    len =     1908   nex =        5

     Term   12350   12052        -         0
     Intr   12644   12437        -        0
     Intr   13278   12727        -        0
     Intr   13478   13381        -        0
     Init   13959   13755        -        0

>3869075        /31648

    len =     1810    nex =        3

     Init   18786   18937        +        0
     Intr   19201   19479        +        0
     Term   20326   20591        +        0

>3869075        /20274

    len =     1795    nex =        2

     Init   29646   29969        +        0
     Term   30976   31440        +        0

>3869075        /9242

    len =      938    nex =        2

     Init   31907   32136        +        0
     Term   32507   32844        +        0

>3869075        /25655

    len =      896    nex =        2

     Init   34505   34737        +        0
     Term   35146   35400        +        0

>3869075        /26281

    len =      894    nex =        2

     Init   34509   34737        +        0
     Term   35146   35402        +        0

>3869075        /3657

    len =      490    nex =        1

     Sngl   36909   37394        +        0

>3869075        /30416

    len =     2410    nex =        7

     Term   62622   62304        -        0
     Intr   62778   62704        -        0
     Intr   63019   62876        -        0
     Intr   63302   63104        -        0
     Intr   63790   63564        -        0
     Intr   63930   63882        -        0
     Init   64704   64524        -        0

>3869075        /1728
```

```
len =    1844   nex =       5

 Init   71156   71325       +        0
 Intr   71732   71877       +        0
 Intr   71985   72203       +        0
 Intr   72292   72504       +        0
 Term   72590   72999       +        0

>3869075        /42640

len =    1307   nex =       4

 Init   71693   71877       +        0
 Intr   71985   72203       +        0
 Intr   72292   72504       +        0
 Term   72590   72999       +        0

>3869075        /33687

len =    1549   nex =       2

 Init    7633    8264       +        0
 Term    8616    8707       +        0

>3873174        /148790

len =    1397   nex =       5

 Term   27243   26811       -        0
 Intr   27470   27381       -        0
 Intr   27601   27558       -        0
 Intr   27797   27701       -        0
 Init   28207   27989       -        0

>3873174        /101691

len =     670   nex =       3

 Term   27601   27558       -        0
 Intr   27797   27701       -        0
 Init   28227   27989       -        0

>3873174        /38697

len =    1472   nex =       5

 Term   27243   26768       -        0
 Intr   27470   27381       -        0
 Intr   27601   27558       -        0
 Intr   27797   27701       -        0
 Init   28239   27989       -        0

>3873174        /27203

len =     776   nex =       1

 Sngl   35931   36706       +        0

>3873174        /23916
```

```
  len =     635   nex =        1

   Sngl    3852    3218         -        0

>3873174        /106667

  len =     572   nex =        1

   Sngl    3852    3281         -        0

>3873174        /7438

  len =    1037   nex =        1

   Sngl   45314   46350         +        0

>3873174        /2558

  len =    1620   nex =        1

   Sngl    6341    7960         +        0

>3873174        /40058

  len =    2300   nex =        5

   Term   62162   61975         -        0
   Intr   62557   62385         -        0
   Intr   63236   63043         -        0
   Intr   63711   63642         -        0
   Init   64274   64013         -        0

>3873174        /125844

  len =     747   nex =        1

   Sngl    7214    7960         +        0

>3873174        /37036

  len =    1550   nex =        2

   Term   74393   73816         -        0
   Init   75365   74610         -        0

>3873174        /37030

  len =    2803   nex =        9

   Term   97302   97150         -        0
   Intr   97507   97385         -        0
   Intr   97791   97597         -        0
   Intr   98007   97879         -        0
   Intr   98258   98096         -        0
   Intr   98487   98352         -        0
   Intr   98916   98575         -        0
   Intr   99091   99011         -        0
   Init   99952   99437         -        0
```

```
>3885325        /21742

   len =      790    nex =        3

    Term   19497    19031        -        0
    Intr   19671    19581        -        0
    Init   19813    19745        -        0

>3885325        /37160

   len =     2950    nex =       10

    Term   19497    19026        -        0
    Intr   19671    19581        -        0
    Intr   19813    19745        -        0
    Intr   20265    20078        -        0
    Intr   20516    20382        -        0
    Intr   20681    20607        -        0
    Intr   21008    20768        -        0
    Intr   21560    21482        -        0
    Intr   21743    21651        -        0
    Init   21973    21878        -        0

>3885325        /8342

   len =     2611    nex =       10

    Term   28061    27841        -        0
    Intr   28235    28145        -        0
    Intr   28405    28340        -        0
    Intr   28839    28793        -        0
    Intr   29013    28929        -        0
    Intr   29188    29102        -        0
    Intr   29519    29446        -        0
    Intr   29749    29666        -        0
    Intr   30098    30059        -        0
    Init   30451    30230        -        0

>3885325        /14041

   len =     1182    nex =        2

    Init   31479    31619        +        0
    Term   32162    32660        +        0

>3885325        /29321

   len =      936    nex =        2

    Init   31484    31619        +        0
    Term   32162    32419        +        0

>3885325        /23675

   len =     1196    nex =        2

    Init   31488    31619        +        0
    Term   32162    32683        +        0

>3885325        /7008
```

```
   len =      996    nex =        2

    Init   31541    31619         +         0
    Term   32162    32536         +         0

>3885325        /35542

   len =     4002    nex =        3

    Term   76796    76680         -         0
    Intr   77285    77136         -         0
    Init   77644    77555         -         0

>3885325        /8450

   len =     1248    nex =        3

    Init   94767    95217         +         0
    Intr   95576    95626         +         0
    Term   95755    96014         +         0

>3885325        /30446

   len =      942    nex =        2

    Term   98533    97856         -         0
    Init   98797    98628         -         0

>3892698        /5111

   len =      852    nex =        1

    Sngl   49208    48945         -         0

>3892698        /35338

   len =     2170    nex =        2

    Term   47914    47683         -         0
    Init   49208    49126         -         0

>3892698        /36849

   len =      772    nex =        1

    Sngl   51041    50270         -         0

>3892698        /18749

   len =     1672    nex =        2

    Term   56961    55850         -         0
    Init   57521    57295         -         0

>3892698        /11009

   len =     3236    nex =        6

    Term   54492    54297         -         0
```

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```
    Intr   54670   54612        -        0
    Intr   54925   54776        -        0
    Intr   55085   54993        -        0
    Intr   56961   56851        -        0
    Init   57532   57295        -        0

>3892698        /5348

    len =     535   nex =        1

    Sngl   74771   74237        -        0

>3894156        /11351

    len =    2097   nex =        4

    Init   37638   37696        +        0
    Intr   37801   38037        +        0
    Intr   38134   38351        +        0
    Term   38433   38887        +        0

>3894156        /18246

    len =    2050   nex =        9

    Init   44721   44808        +        0
    Intr   44904   44960        +        0
    Intr   45125   45231        +        0
    Intr   45327   45398        +        0
    Intr   45557   45610        +        0
    Intr   45697   45772        +        0
    Intr   45919   45980        +        0
    Intr   46137   46196        +        0
    Term   46455   46618        +        0

>3894156        /107424

    len =    2332   nex =        9

    Init   44688   44808        +        0
    Intr   44904   44960        +        0
    Intr   45125   45231        +        0
    Intr   45327   45398        +        0
    Intr   45557   45610        +        0
    Intr   45697   45772        +        0
    Intr   45919   45980        +        0
    Intr   46137   46196        +        0
    Term   46455   47019        +        0

>3894156        /114042

    len =    1556   nex =        2

    Term   47511   46634        -        0
    Init   48189   47592        -        0

>3894156        /38635

    len =    2276   nex =        5
```

```
     Init   68187   68540        +        0
     Intr   68715   68747        +        0
     Intr   68929   69157        +        0
     Intr   69743   69869        +        0
     Term   69944   70462        +        0

  >3894156       /41104

     len =    1762   nex =       5

     Init   68444   68540        +        0
     Intr   68715   68747        +        0
     Intr   68929   69157        +        0
     Intr   69743   69869        +        0
     Term   69944   70205        +        0

  >3894179       /36320

     len =     158   nex =       1

     Sngl   19935   19778        -        0

  >3894179       /13608

     len =    1419   nex =       3

     Init    3877    4048        +        0
     Intr    4194    4398        +        0
     Term    5037    5295        +        0

  >3894179       /11854

     len =    2214   nex =       6

     Term   38528   38322        -        0
     Intr   38784   38647        -        0
     Intr   39404   39281        -        0
     Intr   39652   39529        -        0
     Intr   39874   39742        -        0
     Init   40535   40056        -        0

  >3894179       /125677

     len =     676   nex =       2

     Init    6794    6915        +        0
     Term    7018    7469        +        0

  >3894179       /9236

     len =     575   nex =       1

     Sngl   76198   76772        +        0

  >3894179       /15522

     len =     527   nex =       1

     Sngl   76584   76058        -        0
```

```
>3894179        /31116

    len =      459    nex =        1

     Sngl   80390    80848          +        0

>3894179        /30800

    len =      409    nex =        1

     Sngl   83631    83223          -        0

>3894179        /37949

    len =     1100    nex =        4

     Term   83667    83218          -        0
     Intr   83835    83752          -        0
     Intr   83992    83914          -        0
     Init   84302    84098          -        0

>3894179        /11537

    len =      953    nex =        1

     Sngl    8992     9944          +        0

>3927822        /32642

    len =      500    nex =        1

     Sngl    1179      680          -        0

>3927822        /2058

    len =      927    nex =        1

     Sngl   26615    25689          -        0

>3927822        /35476

    len =      589    nex =        2

     Term   28078    27741          -        0
     Init   28329    28179          -        0

>3927822        /31457

    len =     2687    nex =       10

     Term   28078    27729          -        0
     Intr   28328    28179          -        0
     Intr   28740    28637          -        0
     Intr   28940    28833          -        0
     Intr   29186    29082          -        0
     Intr   29361    29288          -        0
     Intr   29506    29446          -        0
     Intr   29658    29584          -        0
     Intr   29879    29769          -        0
     Init   30415    29968          -        0
```

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```
>3927822        /29679

  len =    2351   nex =        8

   Init   49567   49905        +         0
   Intr   49994   50053        +         0
   Intr   50131   50202        +         0
   Intr   50276   50397        +         0
   Intr   50479   50619        +         0
   Intr   50710   50817        +         0
   Intr   50893   51145        +         0
   Term   51224   51814        +         0

>3927822        /22382

  len =     660   nex =        1

   Sngl   66099   65440        -         0

>3927822        /10159

  len =    1250   nex =        6

   Term   85753   85484        -         0
   Intr   85900   85833        -         0
   Intr   86011   85978        -         0
   Intr   86139   86108        -         0
   Intr   86442   86391        -         0
   Init   86733   86530        -         0

>3927822        /948

  len =    1606   nex =        1

   Sngl   88569   86964        -         0

>3928074        /7805

  len =    2278   nex =        5

   Init   10584   10991        +         0
   Intr   11688   11817        +         0
   Intr   12087   12157        +         0
   Intr   12246   12326        +         0
   Term   12682   12861        +         0

>3928074        /17830

  len =     819   nex =        2

   Init   15343   15421        +         0
   Term   15807   16161        +         0

>3928074        /38129

  len =    1666   nex =        4

   Term    5915    5673        -         0
   Intr    6499    6134        -         0
```

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```
        Intr     7048     6578          -          0
        Init     7338     7135          -          0

     >3928074        /39558

       len =      2141    nex =        3

        Init    75510    76133          +          0
        Intr    76482    76604          +          0
        Term    76976    77204          +          0

     >3928074        /20810

       len =      1514    nex =        5

        Term    79125    78844          -          0
        Intr    79336    79217          -          0
        Intr    79585    79475          -          0
        Intr    79927    79717          -          0
        Init    80038    80002          -          0

     >3928074        /11662

       len =       898    nex =        2

        Term     8846     8488          -          0
        Init     9385     9139          -          0

     >3980374        /34873

       len =       738    nex =        2

        Term   116215   115949          -          0
        Init   116686   116328          -          0

     >3980374        /17240

       len =      1948    nex =        3

        Init    13701    14199          +          0
        Intr    14385    14654          +          0
        Term    14795    15648          +          0

     >3980374        /13720

       len =      1219    nex =        3

        Term    16100    15850          -          0
        Intr    16298    16222          -          0
        Init    17068    16911          -          0

     >3980374        /5112

       len =      1528    nex =        5

        Init    17481    17533          +          0
        Intr    17782    17834          +          0
        Intr    18312    18350          +          0
        Intr    18477    18546          +          0
        Term    18662    18881          +          0
```

```
>3980374        /25736

  len =    1510   nex =       5

   Init   17406   17533        +        0
   Intr   17782   17834        +        0
   Intr   18312   18350        +        0
   Intr   18477   18546        +        0
   Term   18662   18914        +        0

>3980374        /25828

  len =     691   nex =       1

   Sngl   25933   26623        +        0

>3980374        /38727

  len =     950   nex =       2

   Init   27265   27698        +        0
   Term   27792   28214        +        0

>3980374        /6528

  len =    1035   nex =       2

   Init   34104   34489        +        0
   Term   34608   35138        +        0

>3980374        /24361

  len =     898   nex =       2

   Init   40839   41193        +        0
   Term   41271   41736        +        0

>3980374        /39038

  len =     981   nex =       3

   Init    4299    4497        +        0
   Intr    4750    4890        +        0
   Term    5212    5279        +        0

>3980374        /6157

  len =     741   nex =       2

   Init   45228   45692        +        0
   Term   45776   45968        +        0

>3980374        /23439

  len =    2314   nex =       7

   Init   46866   46968        +        0
   Intr   47598   47735        +        0
   Intr   47926   48071        +        0
```

```
                                            1286
       Intr    48238    48357       +          0
       Intr    48453    48665       +          0
       Intr    48761    48841       +          0
       Term    48926    49179       +          0

  >3980374          /33426

     len =     1249    nex =        4

       Init    47926    48071       +          0
       Intr    48238    48357       +          0
       Intr    48453    48665       +          0
       Term    48761    48842       +          0

  >3980374          /2618

     len =     1394    nex =        5

       Term    54808    54506       -          0
       Intr    55007    54901       -          0
       Intr    55303    55087       -          0
       Intr    55595    55388       -          0
       Init    55899    55798       -          0

  >3980374          /14555

     len =     1516    nex =        5

       Term    57704    57309       -          0
       Intr    57909    57803       -          0
       Intr    58204    57988       -          0
       Intr    58508    58301       -          0
       Init    58824    58614       -          0

  >3980374          /1637

     len =     1285    nex =        5

       Term    66552    66306       -          0
       Intr    66749    66643       -          0
       Intr    67079    66860       -          0
       Intr    67374    67167       -          0
       Init    67590    67476       -          0

  >3980374          /342

     len =     1796    nex =        4

       Init     8035     8458       +          0
       Intr     8947     9099       +          0
       Intr     9183     9280       +          0
       Term     9375     9830       +          0

  >3980374          /28554

     len =     1450    nex =        4

       Init     8140     8458       +          0
       Intr     8947     9099       +          0
       Intr     9183     9280       +          0
```

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```
      Term    9375    9586          +         0

   >3980374        /21655

      len =      310    nex =         1

      Sngl    9418    9723          +         0

   >3983533        /40760

      len =      811    nex =         2

      Init   55705   55965          +         0
      Term   56057   56515          +         0

   >3985931        /11375

      len =     1235    nex =         4

      Term   27019   26645          -         0
      Intr   27179   27114          -         0
      Intr   27323   27258          -         0
      Init   27879   27598          -         0

   >3985931        /32856

      len =     1429    nex =         1

      Sngl   40537   39109          -         0

   >3985931        /4623

      len =     2005    nex =         9

      Term   43824   43509          -         0
      Intr   43989   43913          -         0
      Intr   44159   44065          -         0
      Intr   44295   44251          -         0
      Intr   44544   44399          -         0
      Intr   44741   44654          -         0
      Intr   44873   44843          -         0
      Intr   45006   44981          -         0
      Init   45513   45090          -         0

   >3985931        /109141

      len =      670    nex =         1

      Sngl   73609   72960          -         0

   >3985931        /19506

      len =      692    nex =         1

      Sngl    9471   10162          +         0

   >3985931        /119485

      len =      629    nex =         1
      Sngl     9473    10101          +         0

   >3985931          /4831

      len =       565    nex =         1

      Sngl     9591    10155          +         0

   >3985933          /39005

      len =      4007    nex =        11

      Init    28319    28431          +         0
      Intr    28805    28887          +         0
      Intr    28996    29125          +         0
      Intr    29224    29303          +         0
      Intr    29443    29529          +         0
      Intr    29623    29747          +         0
      Intr    29839    29899          +         0
      Intr    30132    30209          +         0
      Intr    30301    30406          +         0
      Intr    30490    30635          +         0
      Term    30833    31065          +         0

   >3985934          /26805

      len =       677    nex =         1

      Sngl    19613    18937          -         0

   >3985934          /25275

      len =      2482    nex =         9

      Init    37180    37552          +         0
      Intr    37890    38045          +         0
      Intr    38268    38336          +         0
      Intr    38434    38604          +         0
      Intr    38687    38830          +         0
      Intr    38925    39083          +         0
      Intr    39196    39261          +         0
      Intr    39362    39421          +         0
      Term    39502    39661          +         0

   >3985934          /91760

      len =      1371    nex =         6

      Init    38434    38604          +         0
      Intr    38687    38830          +         0
      Intr    38925    39083          +         0
      Intr    39196    39261          +         0
      Intr    39362    39421          +         0
      Term    39502    39658          +         0

   >3985934          /39478

      len =      3370    nex =         4

      Init     3984     4155          +         0
```

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```
      Intr     4879     5952          +         0
      Intr     6176     6354          +         0
      Term     6691     7351          +         0

  >3985934        /31667

     len =     1184    nex =        2

      Init    40094    40516          +         0
      Term    40861    41277          +         0

  >3985934        /118260

     len =      926    nex =        2

      Init    40294    40516          +         0
      Term    40861    41219          +         0

  >3985934        /13962

     len =      568    nex =        1

      Sngl    42221    41654          -         0

  >3985934        /32925

     len =      447    nex =        1

      Sngl    43700    43254          -         0

  >3985934        /14816

     len =     1140    nex =        4

      Init    48454    48662          +         0
      Intr    48749    48979          +         0
      Intr    49063    49263          +         0
      Term    49374    49593          +         0

  >3985949        /158734

     len =      624    nex =        1

      Sngl    10055    10678          +         0

  >3985949        /15880

     len =     1150    nex =        2

      Term    19933    19551          -         0
      Init    20691    20400          -         0

  >3985949        /17909

     len =     3262    nex =        8

      Init    51683    52348          +         0
      Intr    52522    53058          +         0
      Intr    53154    53240          +         0
      Intr    53329    53399          +         0
     Intr   53470   53546        +        0
     Intr   53652   53799        +        0
     Intr   53897   54058        +        0
     Term   54365   54944        +        0

  >3985949       /26535

     len =     1492   nex =       5

     Init   62051   62540        +        0
     Intr   62742   62879        +        0
     Intr   62957   63043        +        0
     Intr   63118   63185        +        0
     Term   63285   63542        +        0

  >3985950       /102285

     len =      436   nex =       1

     Sngl   22343   22778        +        0

  >3985952       /9393

     len =     3254   nex =       6

     Init   12815   13266        +        0
     Intr   13492   13653        +        0
     Intr   13771   13957        +        0
     Intr   14238   14663        +        0
     Intr   15037   15444        +        0
     Term   15529   16068        +        0

  >3985952       /11984

     len =     2739   nex =      12

     Init   20557   20824        +        0
     Intr   20898   20956        +        0
     Intr   21064   21139        +        0
     Intr   21217   21288        +        0
     Intr   21367   21454        +        0
     Intr   21528   21780        +        0
     Intr   21897   22012        +        0
     Intr   22111   22331        +        0
     Intr   22422   22453        +        0
     Intr   22546   22648        +        0
     Intr   22763   22943        +        0
     Term   23026   23295        +        0

  >3985952       /207193

     len =      204   nex =       1

     Sngl   23109   23312        +        0

  >3985952       /3151

     len =      537   nex =       2

     Term   40208   40095        -        0
```

```
    Init    40631   40491         -        0

>3985952        /113501

    len =     910   nex =       2

    Term    40208   39985         -        0
    Init    40888   40491         -        0

>3985952        /38966

    len =    1279   nex =       2

    Term    40208   40095         -        0
    Init    41373   40491         -        0

>3985952        /35731

    len =    2127   nex =      10

    Term    41847   41632         -        0
    Intr    41987   41928         -        0
    Intr    42146   42067         -        0
    Intr    42309   42227         -        0
    Intr    42525   42409         -        0
    Intr    42673   42596         -        0
    Intr    42917   42848         -        0
    Intr    43184   43072         -        0
    Intr    43373   43272         -        0
    Init    43758   43458         -        0

>3985952        /2064

    len =    2367   nex =       5

    Init    49630   49929         +        0
    Intr    50622   50752         +        0
    Intr    51041   51168         +        0
    Intr    51324   51530         +        0
    Term    51678   51996         +        0

>3985952        /41430

    len =    2159   nex =       6

    Init    52202   52485         +        0
    Intr    52919   53097         +        0
    Intr    53191   53274         +        0
    Intr    53631   53700         +        0
    Intr    53796   53929         +        0
    Term    54020   54360         +        0

>3985952        /7925

    len =    1873   nex =       1

    Sngl    61124   62996         +        0

>3985952        /12726
```

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```
   len =      291    nex =        1

    Sngl   62743    63033          +         0

>3985952          /6129

   len =      629    nex =        1

    Sngl   70947    71575          +         0

>3985954          /850

   len =     2781    nex =        9

    Term   22960    22894          -         0
    Intr   23249    23031          -         0
    Intr   23819    23641          -         0
    Intr   24255    24075          -         0
    Intr   24624    24497          -         0
    Intr   24745    24704          -         0
    Intr   24912    24833          -         0
    Intr   25199    25105          -         0
    Init   25674    25295          -         0

>3985954          /39002

   len =     2234    nex =        3

    Init   51257    51740          +         0
    Intr   51829    52098          +         0
    Term   52600    53490          +         0

>3985955          /33506

   len =     2022    nex =        6

    Term   12286    12055          -         0
    Intr   12479    12381          -         0
    Intr   12746    12565          -         0
    Intr   12936    12836          -         0
    Intr   13257    13097          -         0
    Init   14076    13714          -         0

>3985955          /151935

   len =      175    nex =        1

    Sngl   55158    55332          +         0

>3985955          /22697

   len =     1494    nex =        6

    Term   56280    55912          -         0
    Intr   56493    56416          -         0
    Intr   56671    56580          -         0
    Intr   56947    56897          -         0
    Intr   57238    57184          -         0
    Init   57405    57326          -         0
```

```
>3985955        /42952

   len =     1548    nex =        4

    Init       61      157          +         0
    Intr      630      706          +         0
    Intr      796      868          +         0
    Term      974     1608          +         0

>3985955        /33522

   len =     1783    nex =        0

>3985957        /108603

   len =      472    nex =        1

    Sngl    14977    15448          +         0

>3985957        /38105

   len =     2110    nex =        6

    Init     2224     2751          +         0
    Intr     3237     3409          +         0
    Intr     3511     3559          +         0
    Intr     3661     3827          +         0
    Intr     3920     4003          +         0
    Term     4106     4329          +         0

>3985957        /99783

   len =      770    nex =        1

    Sngl    32397    33166          +         0

>3985957        /35856

   len =     2121    nex =        6

    Init    51112    51715          +         0
    Intr    52102    52204          +         0
    Intr    52327    52415          +         0
    Intr    52619    52710          +         0
    Intr    52792    52889          +         0
    Term    52976    53232          +         0

>3985958        /36809

   len =     1903    nex =        4

    Init    32457    32776          +         0
    Intr    32856    32932          +         0
    Intr    33601    33919          +         0
    Term    33996    34359          +         0

>3985958        /36602

   len =     2296    nex =        5
   Init    5089    5425        +       0
   Intr    6229    6296        +       0
   Intr    6383    6514        +       0
   Intr    6654    6769        +       0
   Term    6876    7384        +       0

>3985958       /25325

  len =    1821    nex =       6

   Init   52395   52503        +       0
   Intr   53039   53117        +       0
   Intr   53241   53312        +       0
   Intr   53437   53619        +       0
   Intr   53708   53783        +       0
   Term   53955   54215        +       0

>3985958       /30108

  len =     983    nex =       4

   Term   61360   61218        -       0
   Intr   61713   61665        -       0
   Intr   61976   61828        -       0
   Init   62200   62078        -       0

>3985958       /11295

  len =    1274    nex =       4

   Term   73348   72789        -       0
   Intr   73499   73432        -       0
   Intr   73829   73580        -       0
   Init   74062   73922        -       0

>3985958       /17636

  len =     585    nex =       1

   Sngl    7885    7301        -       0

>3985958       /16784

  len =    1046    nex =       2

   Term   80867   80492        -       0
   Init   81076   80939        -       0

>3985958       /97883

  len =    1074    nex =       4

   Init    8598    8696        +       0
   Intr    8960    9035        +       0
   Intr    9202    9513        +       0
   Term    9628    9671        +       0

>4003353       /11111

  len =    1241    nex =       4
```

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```
        Term    18714    18255          -         0
        Intr    18983    18813          -         0
        Intr    19249    19172          -         0
        Init    19495    19337          -         0

  >4003353        /465

      len =      717    nex =        2

        Init    23467    23626          +         0
        Term    23709    24183          +         0

  >4003353        /22440

      len =     2230    nex =        3

        Term    46405    45777          -         0
        Intr    46873    46618          -         0
        Init    48003    47263          -         0

  >4003353        /12261

      len =     2365    nex =        3

        Term    46405    45643          -         0
        Intr    46873    46618          -         0
        Init    47683    47263          -         0

  >4003353        /21954

      len =     2197    nex =        8

        Term     3077     2689          -         0
        Intr     3298     3253          -         0
        Intr     3684     3577          -         0
        Intr     3834     3775          -         0
        Intr     4211     4130          -         0
        Intr     4401     4286          -         0
        Intr     4622     4494          -         0
        Init     4885     4714          -         0

  >4003353        /39309

      len =     2590    nex =        8

        Init    69646    69794          +         0
        Intr    69914    70187          +         0
        Intr    70279    70334          +         0
        Intr    70442    70590          +         0
        Intr    70894    71049          +         0
        Intr    71189    71234          +         0
        Intr    71571    71637          +         0
        Term    71818    71894          +         0

  >4003353        /155178

      len =      452    nex =        1

        Sngl    72936    72485          -         0
```

```
>4003353        /33702

   len =      298   nex =       1

     Sngl   73011   72714        -         0

>4003353        /21040

   len =      511   nex =       1

     Sngl   73016   72506        -         0

>4006815        /11214

   len =     2790   nex =      10

     Init  107144  107253        +         0
     Intr  107333  107388        +         0
     Intr  107462  107594        +         0
     Intr  107672  107746        +         0
     Intr  107835  107950        +         0
     Intr  108022  108073        +         0
     Intr  108174  108225        +         0
     Intr  108306  108363        +         0
     Intr  108456  108554        +         0
     Term  108635  108985        +         0

>4006815        /36277

   len =     2791   nex =      10

     Init  107144  107253        +         0
     Intr  107333  107388        +         0
     Intr  107462  107594        +         0
     Intr  107672  107746        +         0
     Intr  107835  107950        +         0
     Intr  108022  108073        +         0
     Intr  108174  108225        +         0
     Intr  108306  108363        +         0
     Intr  108456  108554        +         0
     Term  108635  108992        +         0

>4006815        /10529

   len =     1092   nex =       0

>4006815        /2886

   len =      624   nex =       1

     Sngl  114766  115389        +         0

>4006815        /36965

   len =     2350   nex =       9

     Init   13554   13743        +         0
     Intr   13824   13879        +         0
     Intr   13954   14112        +         0
```

```
  Intr   14196   14295          +          0
  Intr   14381   14523          +          0
  Intr   14714   14819          +          0
  Intr   15089   15227          +          0
  Intr   15323   15591          +          0
  Term   15672   15895          +          0

>4006815       /2240

 len =     2135    nex =        9

  Init   18070   18214          +          0
  Intr   18297   18391          +          0
  Intr   18473   18554          +          0
  Intr   18619   18681          +          0
  Intr   18791   18845          +          0
  Intr   18932   19014          +          0
  Intr   19321   19416          +          0
  Intr   19606   19665          +          0
  Term   19874   20200          +          0

>4006815       /18652

 len =     2128    nex =        9

  Init   18079   18214          +          0
  Intr   18297   18391          +          0
  Intr   18473   18554          +          0
  Intr   18619   18715          +          0
  Intr   18791   18845          +          0
  Intr   18932   19014          +          0
  Intr   19321   19416          +          0
  Intr   19606   19665          +          0
  Term   19874   20202          +          0

>4006815       /29981

 len =      550    nex =        1

  Sngl   47608   47066          -          0

>4006815       /12018

 len =      594    nex =        1

  Sngl   48483   47890          -          0

>4006815       /33863

 len =      207    nex =        1

  Sngl   53635   53429          -          0

>4006815       /6541

 len =     1765    nex =        2

  Term   54408   53438          -          0
  Init   55202   54521          -          0
```

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```
>4006885        /38141

   len =    1902   nex =        3

    Init  102102  102792        +        0
    Intr  103399  103488        +        0
    Term  103569  103636        +        0

>4006885        /21999

   len =    1291   nex =        2

    Init  107190  107560        +        0
    Term  107858  108480        +        0

>4006885        /143475

   len =    1163   nex =        2

    Term  113830  113699        -        0
    Init  114861  114488        -        0

>4006885        /36845

   len =    1815   nex =        7

    Init  127608  127748        +        0
    Intr  127830  127937        +        0
    Intr  128025  128163        +        0
    Intr  128267  128437        +        0
    Intr  128516  128756        +        0
    Intr  128833  128924        +        0
    Term  129014  129422        +        0

>4006885        /95065

   len =     238   nex =        1

    Sngl  143397  143160        -        0

>4006885        /40968

   len =    1059   nex =        6

    Term  166624  166434        -        0
    Intr  166800  166712        -        0
    Intr  166946  166883        -        0
    Intr  167137  167035        -        0
    Intr  167317  167214        -        0
    Init  167492  167421        -        0

>4006885        /36701

   len =    1410   nex =        1

    Sngl   26044   24635        -        0

>4006885        /30175

   len =    1665   nex =        5
```

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```
     Init    27294    27381          +         0
     Intr    27469    27545          +         0
     Intr    27672    27749          +         0
     Intr    27847    27963          +         0
     Term    28043    28323          +         0

  >4006885        /17161

     len =     564    nex =        0

  >4006885        /39745

     len =    2077    nex =        4

     Term    31714    31054          -         0
     Intr    31984    31812          -         0
     Intr    32362    32067          -         0
     Init    32732    32435          -         0

  >4006885        /35084

     len =    1873    nex =        2

     Term    43430    42975          -         0
     Init    44847    44183          -         0

  >4006885        /13446

     len =    1002    nex =        4

     Init    74420    74452          +         0
     Intr    74579    74714          +         0
     Intr    74988    75055          +         0
     Term    75142    75421          +         0

  >4027862        /13725

     len =    1630    nex =        3

     Init    13479    13828          +         0
     Intr    14535    14558          +         0
     Term    14658    15106          +         0

  >4027862        /37455

     len =    1792    nex =        5

     Init    19304    19667          +         0
     Intr    19757    19881          +         0
     Intr    20420    20511          +         0
     Intr    20639    20725          +         0
     Term    20814    21095          +         0

  >4027862        /14541

     len =    1376    nex =        4

     Init    39417    39585          +         0
     Intr    39658    39884          +         0
```

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```
        Intr    40036    40369         +         0
        Term    40452    40792         +         0

    >4038029        /12043

      len =     1878    nex =       11

        Term    23390    23308         -         0
        Intr    23553    23476         -         0
        Intr    23748    23695         -         0
        Intr    23896    23833         -         0
        Intr    24208    24163         -         0
        Intr    24367    24295         -         0
        Intr    24518    24447         -         0
        Intr    24700    24608         -         0
        Intr    24842    24790         -         0
        Intr    25011    24921         -         0
        Init    25171    25089         -         0

    >4038029        /12568

      len =     2394    nex =       11

        Term    23390    23002         -         0
        Intr    23553    23476         -         0
        Intr    23748    23695         -         0
        Intr    23896    23833         -         0
        Intr    24208    24163         -         0
        Intr    24367    24295         -         0
        Intr    24518    24447         -         0
        Intr    24700    24608         -         0
        Intr    24842    24790         -         0
        Intr    25011    24921         -         0
        Init    25395    25089         -         0

    >4038029        /31175

      len =      719    nex =        2

        Term    41247    40834         -         0
        Init    41552    41441         -         0

    >4038029        /205695

      len =      575    nex =        2

        Term    46021    45667         -         0
        Init    46241    46125         -         0

    >4038029        /364

      len =      598    nex =        2

        Term    46021    45663         -         0
        Init    46260    46125         -         0

    >4038029        /6995

      len =      767    nex =        2
```

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```
     Term   57543   57164        -        0
     Init   57930   57799        -        0

>4038029        /9804

     len =    2193   nex =        3

     Init   65054   65282        +        0
     Intr   65599   65995        +        0
     Term   66507   66778        +        0

>4049332        /31275

     len =    1090   nex =        1

     Sngl   36708   37796        +        0

>4049332        /12232

     len =    1707   nex =        3

     Init   38921   39520        +        0
     Intr   39710   39871        +        0
     Term   40057   40627        +        0

>4049332        /31460

     len =    1016   nex =        2

     Init   39710   39871        +        0
     Term   40057   40719        +        0

>4049332        /37529

     len =    2144   nex =        3

     Term   63854   63126        -        0
     Intr   64463   63966        -        0
     Init   65269   65079        -        0

>4049332        /34310

     len =    1773   nex =        4

     Term   70001   69714        -        0
     Intr   70410   70363        -        0
     Intr   71182   71025        -        0
     Init   71486   71294        -        0

>4049332        /28012

     len =    1066   nex =        2

     Init   77171   77322        +        0
     Term   77644   78233        +        0

>4049332        /16844

     len =     957   nex =        3
```

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```
    Term   90208   89783        -        0
    Intr   90525   90453        -        0
    Init   90723   90633        -        0

>4056429        /33126

   len =     1510    nex =        5

    Init   10968   11580        +        0
    Intr   11710   11780        +        0
    Intr   11934   12053        +        0
    Intr   12141   12259        +        0
    Term   12393   12475        +        0

>4056429        /3465

   len =     1407    nex =        4

    Term   18080   17627        -        0
    Intr   18319   18176        -        0
    Intr   18697   18419        -        0
    Init   19033   18817        -        0
>4056429        /26826

   len =     1648    nex =        5

    Term   27443   26816        -        0
    Intr   27669   27526        -        0
    Intr   27875   27753        -        0
    Intr   28125   27964        -        0
    Init   28463   28218        -        0

>4056429        /92839

   len =     1320    nex =        4

    Term   29933   29790        -        0
    Intr   30139   30074        -        0
    Intr   30433   30254        -        0
    Init   30662   30509        -        0

>4056429        /6245

   len =     1515    nex =        5

    Term   33847   33332        -        0
    Intr   34084   33941        -        0
    Intr   34319   34185        -        0
    Intr   34571   34422        -        0
    Init   34846   34705        -        0

>4056429        /10721

   len =     2590    nex =        4

    Term   47922   47487        -        0
    Intr   48181   48035        -        0
    Intr   49877   49731        -        0
    Init   50069   49958        -        0
```

```
>4056429        /36437

   len =    1094   nex =        1

    Sngl   59727   60545         +        0

>4056429        /15450

   len =    1313   nex =        2

    Init   74846   75064         +        0
    Term   75185   75669         +        0

>4056429        /18845

   len =    2571   nex =        9

    Init   94527   94779         +        0
    Intr   94873   94949         +        0
    Intr   95041   95163         +        0
    Intr   95819   95923         +        0
    Intr   96020   96092         +        0
    Intr   96192   96263         +        0
    Intr   96369   96521         +        0
    Intr   96649   96772         +        0
    Term   96860   97097         +        0

>4056476        /31973

   len =     570   nex =        1

    Sngl  105977  106546         +        0

>4056476        /39206

   len =    1908   nex =        4

    Init  107066  107469         +        0
    Intr  107817  107846         +        0
    Intr  107898  108112         +        0
    Term  108463  108973         +        0

>4056476        /205753

   len =      99   nex =        1

    Sngl  107310  107212         -        0

>4056476        /98881

   len =    1823   nex =        9

    Term    9508    9155         -        0
    Intr    9685    9625         -        0
    Intr    9877    9786         -        0
    Intr   10054    9975         -        0
    Intr   10183   10147         -        0
    Intr   10330   10271         -        0
    Intr   10462   10417         -        0
    Intr   10672   10540         -        0
    Init    10977   10757         -        0

>4056476        /15831

    len =     1631    nex =        4

    Init    11178   11501         +        0
    Intr    11809   11949         +        0
    Intr    12030   12302         +        0
    Term    12389   12808         +        0

>4056476        /11642

    len =     1479    nex =        3

    Term  112566   112276         -        0
    Intr  112710   112642         -        0
    Init  113754   113600         -        0

>4056476        /29782

    len =     1167    nex =        4

    Term    17151   17095         -        0
    Intr    17449   17246         -        0
    Intr    17819   17548         -        0
    Init    18261   18164         -        0

>4056476        /8397

    len =     1454    nex =        3

    Term    17151   16982         -        0
    Intr    17449   17246         -        0
    Init    17819   17548         -        0

>4056476        /33020

    len =     1690    nex =        6

    Term    39931   39868         -        0
    Intr    40166   40071         -        0
    Intr    40433   40246         -        0
    Intr    40634   40539         -        0
    Intr    40814   40775         -        0
    Init    41554   41439         -        0

>4056476        /113469

    len =     2530    nex =        6

    Term    39931   39376         -        0
    Intr    40166   40071         -        0
    Intr    40433   40246         -        0
    Intr    40634   40539         -        0
    Intr    40814   40775         -        0
    Init    41552   41439         -        0

>4056476        /43008
```

```
  len =     2506    nex =        6

   Term    39931    39406         -          0
   Intr    40166    40071         -          0
   Intr    40433    40246         -          0
   Intr    40634    40539         -          0
   Intr    40814    40775         -          0
   Init    41552    41439         -          0

>4056476          /8849

  len =     1775    nex =        4

   Term     4259     4148         -          0
   Intr     4695     4357         -          0
   Intr     5068     4967         -          0
   Init     5605     5183         -          0

>4056476          /36303

  len =     1786    nex =        4

   Term     4259     4148         -          0
   Intr     4695     4357         -          0
   Intr     5068     4967         -          0
   Init     5616     5183         -          0

>4056476          /9001

  len =     1248    nex =        1

   Sngl    66616    65369         -          0

>4056476          /16393

  len =     2532    nex =        6

   Term    96093    95777         -          0
   Intr    96329    96178         -          0
   Intr    96583    96422         -          0
   Intr    97005    96654         -          0
   Intr    97159    97097         -          0
   Init    98308    98024         -          0

>4063730          /13660

  len =     1755    nex =        3

   Init    60286    60642         +          0
   Intr    61422    61490         +          0
   Term    61594    62040         +          0

>4063730          /21240

  len =     2170    nex =        5

   Init    72484    73175         +          0
   Intr    73451    73571         +          0
   Intr    73660    73844         +          0
   Intr    73922    74053         +          0
```

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```
        Term    74132    74268         +          0

    >4063730         /38879

        len =     2303    nex =          7

        Term    91140    90858         -          0
        Intr    91393    91280         -          0
        Intr    91609    91538         -          0
        Intr    92030    91961         -          0
        Intr    92214    92117         -          0
        Intr    92495    92294         -          0
        Init    93160    92726         -          0

    >4063735         /1351

        len =     2263    nex =          3

        Init   105458   105582         +          0
        Intr   106342   106839         +          0
        Term   106936   107720         +          0

    >4063735         /36464

        len =     1399    nex =          2

        Init   106315   106839         +          0
        Term   106936   107713         +          0

    >4063735         /110447

        len =      659    nex =          1

        Sngl   107104   107748         +          0

    >4063735         /99323

        len =     1093    nex =          1

        Sngl    15861    16953         +          0

    >4063735         /148671

        len =     1079    nex =          2

        Term    43823    43650         -          0
        Init    44402    44347         -          0

    >4063735         /29766

        len =     1949    nex =          6

        Term    97324    96849         -          0
        Intr    97547    97491         -          0
        Intr    97772    97630         -          0
        Intr    98263    98128         -          0
        Intr    98642    98446         -          0
        Init    98797    98734         -          0

    >4063737         /39796
```

```
   len =     2050    nex =        8

    Term     8947     8682         -          0
    Intr     9161     9108         -          0
    Intr     9393     9318         -          0
    Intr     9521     9490         -          0
    Intr     9711     9616         -          0
    Intr     9843     9804         -          0
    Intr     9992     9931         -          0
    Init    10722    10413         -          0

>4063737         /37252

   len =     2858    nex =       12

    Term    33875    33630         -          0
    Intr    34209    33988         -          0
    Intr    34433    34313         -          0
    Intr    34722    34531         -          0
    Intr    34908    34836         -          0
    Intr    35127    35004         -          0
    Intr    35284    35208         -          0
    Intr    35494    35372         -          0
    Intr    35672    35567         -          0
    Intr    35825    35763         -          0
    Intr    36084    35913         -          0
    Init    36487    36329         -          0

>4063737         /36243

   len =      501    nex =        1

    Sngl    56605    57105         +          0

>4063737         /13761

   len =     2567    nex =        2

    Init    61983    62535         +          0
    Term    63541    64549         +          0

>4063737         /17020

   len =     2217    nex =        2

    Init    74589    74633         +          0
    Term    74734    75239         +          0

>4063756         /154687

   len =     1056    nex =        3

    Init    37451    37699         +          0
    Intr    38169    38269         +          0
    Term    38354    38506         +          0

>4063756         /15276

   len =     2125    nex =        4
```

```
     Init   37451   37699        +        0
     Intr   38169   38269        +        0
     Intr   38354   38547        +        0
     Term   38976   39575        +        0

>4079614        /9756

     len =      974    nex =         1

     Sngl   27761   28734        +        0

>4079614        /36980

     len =     1107    nex =         1

     Sngl   38778   37672        -        0

>4079614        /18761

     len =      250    nex =         1

     Sngl   64013   63770        -        0

>4079614        /39763

     len =     1030    nex =         1

     Sngl   73340   74368        +        0

>4079614        /6208

     len =     2548    nex =         5

     Init   75720   75893        +        0
     Intr   75986   76102        +        0
     Intr   76525   76695        +        0
     Intr   76777   76989        +        0
     Term   77081   77542        +        0

>4079614        /4512

     len =      490    nex =         1

     Sngl   96496   96984        +        0

>4092471        /22446

     len =      506    nex =         1

     Sngl   11405   11769        +        0

>4092471        /103691

     len =     1759    nex =         3

     Term   42647   42351        -        0
     Intr   43888   43766        -        0
     Init   43998   43927        -        0
```

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```
>4092471        /20391

    len =    1615   nex =       3

    Term   42647   42495       -        0
    Intr   43888   43766       -        0
    Init   43998   43927       -        0

>4092472        /16473

    len =    1615   nex =       1

    Sngl   20093   20396        +        0

>4092472        /3126

    len =    2865   nex =       5

    Term   23958   23048       -        0
    Intr   24223   24110       -        0
    Intr   24606   24316       -        0
    Intr   24981   24673       -        0
    Init   25912   25060       -        0

>4092472        /104778

    len =    3719   nex =       7

    Term   46430   46119       -        0
    Intr   46578   46521       -        0
    Intr   46706   46670       -        0
    Intr   47519   47278       -        0
    Intr   47978   47862       -        0
    Intr   48234   48193       -        0
    Init   49837   49522       -        0

>4096078        /155459

    len =     415   nex =       1

    Sngl   42191   42304        +        0

>4096078        /37034

    len =    1948   nex =      10

    Term   45801   45724       -        0
    Intr   45988   45893       -        0
    Intr   46149   46063       -        0
    Intr   46287   46219       -        0
    Intr   46556   46369       -        0
    Intr   46697   46640       -        0
    Intr   46828   46769       -        0
    Intr   46936   46901       -        0
    Intr   47134   47023       -        0
    Init   47376   47303       -        0

>4096078        /21724

    len =    2303   nex =      10
```

```
      Term   45801   45724         -         0
      Intr   45988   45893         -         0
      Intr   46149   46063         -         0
      Intr   46287   46219         -         0
      Intr   46556   46369         -         0
      Intr   46697   46640         -         0
      Intr   46828   46769         -         0
      Intr   46936   46901         -         0
      Intr   47134   47023         -         0
      Init   47599   47303         -         0

  >4096078        /15956

     len =     1336   nex =        7

      Init   54120   54197         +         0
      Intr   54285   54356         +         0
      Intr   54439   54543         +         0
      Intr   54618   54710         +         0
      Intr   54798   54890         +         0
      Intr   54983   55106         +         0
      Term   55206   55327         +         0

  >4096078        /8301

     len =     1697   nex =        7

      Term   55725   55556         -         0
      Intr   55873   55814         -         0
      Intr   56063   55943         -         0
      Intr   56218   56142         -         0
      Intr   56400   56306         -         0
      Intr   56619   56513         -         0
      Init   57252   56708         -         0

  >4096078        /20936

     len =     1419   nex =        3

      Term   58407   58007         -         0
      Intr   58821   58569         -         0
      Init   59425   59245         -         0

  >4096078        /11587

     len =      698   nex =        1

      Sngl   62604   61911         -         0

  >4115352        /125955

     len =     1433   nex =        3

      Term   26979   26916         -         0
      Intr   27312   27194         -         0
      Init   27672   27485         -         0

  >4115352        /19338
```

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```
len =    1003   nex =      3

 Term   26979   26681        -        0
 Intr   27312   27194        -        0
 Init   27683   27485        -        0

>4115352        /41682

len =    1450   nex =      2

 Term   27312   27194        -        0
 Init   27690   27485        -        0

>4115370        /104717

len =    1480   nex =      1

 Sngl   21740   21519        -        0

>4115370        /117533

len =    1330   nex =      5

 Term   43085   42601        -        0
 Intr   43330   43160        -        0
 Intr   43471   43406        -        0
 Intr   43806   43554        -        0
 Init   43929   43880        -        0

>4115370        /40540

len =    2671   nex =      9

 Term   86403   86022        -        0
 Intr   86600   86484        -        0
 Intr   87059   86681        -        0
 Intr   87301   87146        -        0
 Intr   87515   87379        -        0
 Intr   87708   87589        -        0
 Intr   87929   87801        -        0
 Intr   88216   88023        -        0
 Init   88692   88422        -        0

>4115912        /2343

len =    2183   nex =      9

 Term   15312   14953        -        0
 Intr   15468   15407        -        0
 Intr   15691   15575        -        0
 Intr   15840   15781        -        0
 Intr   16048   15936        -        0
 Intr   16206   16134        -        0
 Intr   16322   16278        -        0
 Intr   16522   16412        -        0
 Init   17135   16691        -        0

>4115912        /11730

len =     896   nex =      2
```

```
        Init    34905    35153        +        0
        Term    35318    35800        +        0

  >4115912         /8445

        len =     1254    nex =        4

        Init    95230    95390        +        0
        Intr    95475    95562        +        0
        Intr    95755    95879        +        0
        Term    95956    96483        +        0

  >4115930         /40478

        len =     1543    nex =        2

        Term    41954    41542        -        0
        Init    43084    42069        -        0

  >4115930         /13513

        len =     1185    nex =        4

        Init    46052    46100        +        0
        Intr    46383    46693        +        0
        Intr    46778    46829        +        0
        Term    47059    47236        +        0

  >4115930         /39698

        len =     1656    nex =        3

        Init    53288    53526        +        0
        Intr    54255    54411        +        0
        Term    54517    54943        +        0

  >4115930         /9218

        len =     1162    nex =        3

        Init    59597    59737        +        0
        Intr    60190    60346        +        0
        Term    60446    60758        +        0

  >4115930         /3033

        len =     2350    nex =        8

        Term    75735    75445        -        0
        Intr    75948    75854        -        0
        Intr    76111    76037        -        0
        Intr    76336    76251        -        0
        Intr    76789    76738        -        0
        Intr    77027    76953        -        0
        Intr    77177    77111        -        0
        Init    77787    77261        -        0

  >4159699         /1558
```

```
     len =     1272    nex =        2

      Term   14823    14303        -        0
      Init   15574    15206        -        0

>4159699           /17101

     len =     2330    nex =        4

      Init    8693     8791        +        0
      Intr    9044     9107        +        0
      Intr    9186     9395        +        0
      Term    9532    10243        +        0

>4159700           /11277

     len =     2170    nex =       11

      Init    2785     3024        +        0
      Intr    3119     3211        +        0
      Intr    3301     3401        +        0
      Intr    3481     3547        +        0
      Intr    3634     3733        +        0
      Intr    3811     3924        +        0
      Intr    4060     4197        +        0
      Intr    4305     4389        +        0
      Intr    4488     4537        +        0
      Intr    4639     4703        +        0
      Term    4715     4949        +        0

>4159700           /9884

     len =     1278    nex =        2

      Term   33490    33138        -        0
      Init   33894    33573        -        0

>4159700           /106065

     len =      550    nex =        1

      Sngl      36      578        +        0

>4159700           /11225

     len =     1959    nex =        2

      Init    5625     6382        +        0
      Term    6789     7074        +        0

>4159700           /37830

     len =     2006    nex =        2

      Init    5116     6382        +        0
      Term    6789     7121        +        0

>4159700           /39753

     len =     1990    nex =        2
```

```
    Init     5625    6382         +        0
    Term     6789    7121         +        0

>4159702        /39782

   len =     1769    nex =        4

    Term    19118   18896         -        0
    Intr    19546   19462         -        0
    Intr    19766   19651         -        0
    Init    20664   20383         -        0

>4159703        /22343

   len =     2215    nex =        5

    Init     7241    7578         +        0
    Intr     8231    8293         +        0
    Intr     8510    8587         +        0
    Intr     8667    8829         +        0
    Term     8902    9455         +        0

>4159704        /13704

   len =     1335    nex =        4

    Term    11416   11205         -        0
    Intr    11620   11507         -        0
    Intr    11891   11712         -        0
    Init    12357   12143         -        0

>4159704        /21828

   len =     1419    nex =        4

    Term    11416   11151         -        0
    Intr    11620   11507         -        0
    Intr    11891   11712         -        0
    Init    12357   12143         -        0

>4159704        /109598

   len =     1100    nex =        2

    Init     5940    6324         +        0
    Term     6476    7039         +        0

>4159704        /39832

   len =     2092    nex =        7

    Init    71302   71572         +        0
    Intr    71670   71791         +        0
    Intr    71963   72067         +        0
    Intr    72212   72304         +        0
    Intr    72593   72655         +        0
    Intr    72773   72882         +        0
    Term    73080   73393         +        0
```

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```
>4159704        /13305

   len =    2400   nex =       3

    Init   80000   81012       +         0
    Intr   81347   81522       +         0
    Term   81623   82399       +         0

>4159705        /9508

   len =    1639   nex =       3

    Init   28709   28753       +         0
    Intr   29227   29324       +         0
    Term   29566   29821       +         0

>4159705        /114725

   len =     490   nex =       1

    Sngl   73649   73181       -         0

>4159705        /109298

   len =     730   nex =       1

    Sngl   73980   73255       -         0

>4159705        /326

   len =    1716   nex =       1

    Sngl   74641   73196       -         0

>4159705        /37994

   len =    2303   nex =       7

    Init   77530   77634       +         0
    Intr   77709   77959       +         0
    Intr   78103   78229       +         0
    Intr   78328   78601       +         0
    Intr   78834   78966       +         0
    Intr   79353   79439       +         0
    Term   79694   79832       +         0

>4159706        /112601

   len =     690   nex =       1

    Sngl   10748   11437       +         0

>4159706        /16938

   len =    1330   nex =       1

    Sngl   14792   13472       -         0

>4159706        /30320
```

09595326.061500

```
     len =       370    nex =        1

      Sngl    28894    28532          -          0

 >4159706          /112749

     len =       712    nex =        2

      Term    35876    35547          -          0
      Init    36258    35970          -          0

 >4159706          /8290

     len =      1320    nex =        4

      Term    35876    35548          -          0
      Intr    36256    35970          -          0
      Intr    36422    36349          -          0
      Init    36867    36752          -          0

 >4159706          /35999

     len =      1933    nex =        7

      Init    53834    53966          +          0
      Intr    54115    54280          +          0
      Intr    54489    54541          +          0
      Intr    54877    54939          +          0
      Intr    55266    55316          +          0
      Intr    55381    55498          +          0
      Term    55599    55766          +          0

 >4159706          /4581

     len =      1390    nex =        1

      Sngl    58788    60171          +          0

 >4159706          /38468

     len =      1437    nex =        1

      Sngl    65398    66834          +          0

 >4159706          /35981

     len =      1281    nex =        2

      Init    71773    71993          +          0
      Term    72232    73053          +          0

 >4159706          /27978

     len =      1451    nex =        5

      Init    74975    75143          +          0
      Intr    75510    75632          +          0
      Intr    75728    76041          +          0
      Intr    76128    76180          +          0
      Term    76272    76425          +          0
```

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```
>4159707         /207156

    len =      926    nex =        0

>4159707         /41828

    len =      875    nex =        1

    Sngl   15648   15958          +         0

>4159707         /29375

    len =      792    nex =        1

    Sngl   15648   15949          +         0

>4159707         /37081

    len =      827    nex =        2

    Init   15176   15533          +         0
    Term   15648   16002          +         0

>4159707         /18951

    len =      595    nex =        1

    Sngl    1560    2154          +         0

>4159707         /32148

    len =     1343    nex =        1

    Sngl   16092   17434          +         0

>4159707         /25313

    len =     1016    nex =        1

    Sngl   16466   17481          +         0

>4159707         /41182

    len =      593    nex =        1

    Sngl   16874   17456          +         0

>4159707         /28782

    len =     1096    nex =        3

    Init   27447   27699          +         0
    Intr   27790   28154          +         0
    Term   28252   28542          +         0

>4159707         /97742

    len =     1220    nex =        2
```

```
       Init     2818     3153          +         0
       Term     3582     4037          +         0

  >4159707          /42151

      len =      1186    nex =         2

       Init     2848     3153          +         0
       Term     3582     4033          +         0

  >4159707          /2356

      len =      2110    nex =         7

       Term    28857    28597          -         0
       Intr    29050    28952          -         0
       Intr    29395    29294          -         0
       Intr    29617    29493          -         0
       Intr    29826    29753          -         0
       Intr    30352    30211          -         0
       Init    30705    30436          -         0

  >4159707          /21101

      len =       310    nex =         1

       Sngl    41123    40815          -         0

  >4159707          /106867

      len =      1340    nex =         2

       Term    41164    40724          -         0
       Init    41458    41253          -         0

  >4159707          /22328

      len =      1515    nex =         3

       Term    41164    40703          -         0
       Intr    41458    41253          -         0
       Init    42217    42021          -         0

  >4159707          /18636

      len =       190    nex =         1

       Sngl    42223    42042          -         0

  >4159707          /38214

      len =      1822    nex =         3

       Init    58012    58329          +         0
       Intr    58492    58799          +         0
       Term    58885    59833          +         0

  >4159707          /18979

      len =      1890    nex =         3
```

```
    Init   58012   58329        +        0
    Intr   58492   58799        +        0
    Term   58885   59901        +        0

>4159707        /26705

  len =     538   nex =        1

    Sngl   63569   64106        +        0

>4159707        /15303

  len =    1493   nex =        2

    Term   68076   67448        -        0
    Init   68653   68151        -        0

>4159707        /94231

  len =    1630   nex =        3

    Init   78497   78952        +        0
    Intr   79546   79639        +        0
    Term   79732   80120        +        0

>4159708        /35429

  len =     764   nex =        2

    Init   44910   45015        +        0
    Term   45368   45673        +        0

>4159708        /16564

  len =    1359   nex =        5

    Init   61816   62242        +        0
    Intr   62321   62370        +        0
    Intr   62462   62549        +        0
    Intr   62641   62757        +        0
    Term   62943   63174        +        0

>4159708        /39933

  len =    1197   nex =        1

    Sngl   76529   77725        +        0

>4159709        /8209

  len =    1632   nex =        3

    Init   28608   28689        +        0
    Intr   28882   28962        +        0
    Term   29255   29368        +        0

>4159709        /20801

  len =    2026   nex =        8
```

```
     Init    28608    28689          +          0
     Intr    28882    28962          +          0
     Intr    29255    29368          +          0
     Intr    29463    29564          +          0
     Intr    29876    29934          +          0
     Intr    30031    30133          +          0
     Intr    30228    30293          +          0
     Term    30387    30479          +          0

>4159709          /21868

     len =      1990    nex =        3

     Term     1570     1249          -          0
     Intr     2549     2064          -          0
     Init     3231     2810          -          0

>4159709          /13261

     len =      2987    nex =        3

     Term    37003    36228          -          0
     Intr    38163    37674          -          0
     Init    39214    38597          -          0

>4159709          /6579

     len =      2352    nex =        8

     Init    70836    71356          +          0
     Intr    71493    71667          +          0
     Intr    71764    71913          +          0
     Intr    72061    72222          +          0
     Intr    72304    72489          +          0
     Intr    72587    72709          +          0
     Intr    72784    72873          +          0
     Term    72972    73187          +          0

>4159709          /9155

     len =       557    nex =        1

     Sngl    75021    75577          +          0

>4159709          /36834

     len =       657    nex =        2

     Term    76162    75717          -          0
     Init    76373    76339          -          0

>4159709          /115021

     len =       515    nex =        2

     Init    82749    82901          +          0
     Term    83001    83063          +          0

>4159710          /13184
```

```
len =    1870   nex =       7

 Term   14387   14080       -         0
 Intr   14579   14479       -         0
 Intr   14816   14659       -         0
 Intr   14992   14930       -         0
 Intr   15289   15150       -         0
 Intr   15462   15369       -         0
 Init   15947   15591       -         0

>4159710        /100272

len =     670   nex =       1

 Sngl   18553   19215       +         0

>4159711        /18503

len =    1595   nex =       3

 Term   15462   15206       -         0
 Intr   15812   15748       -         0
 Init   16800   16583       -         0

>4159712        /4044

len =     730   nex =       1

 Sngl   10754   10446       -         0

>4159712        /36591

len =    2809   nex =       7

 Term   22587   22215       -         0
 Intr   22740   22669       -         0
 Intr   23033   22830       -         0
 Intr   23401   23272       -         0
 Intr   23755   23529       -         0
 Intr   23937   23881       -         0
 Init   25023   24745       -         0

>4159712        /14490

len =    1090   nex =       3

 Term   74186   73691       -         0
 Intr   74429   74331       -         0
 Init   74774   74516       -         0

>4159712        /1226

len =     794   nex =       2

 Init    8922    9122       +         0
 Term    9221    9715       +         0

>4165340        /12055
```

```
    len =      520    nex =        1

     Sngl  105172  104653         -         0

>4165340        /94038

    len =     2170    nex =        3

     Init     3022     3136        +        0
     Intr     3876     3994        +        0
     Term     4100     4352        +        0

>4165340        /19364

    len =     2182    nex =        3

     Init     3022     3136        +        0
     Intr     3876     3994        +        0
     Term     4100     4372        +        0

>4165340        /38840

    len =     2072    nex =        4

     Init     2281     2871        +        0
     Intr     3022     3136        +        0
     Intr     3876     3994        +        0
     Term     4100     4352        +        0

>4165340        /19740

    len =     2030    nex =        0

>4165340        /11470

    len =     1919    nex =        0

>4165340        /31383

    len =      655    nex =        1

     Sngl     6708     6054         -         0

>4165340        /16005

    len =     2365    nex =       10

     Init    84598    84682        +        0
     Intr    84770    84891        +        0
     Intr    84993    85214        +        0
     Intr    85301    85485        +        0
     Intr    85563    85698        +        0
     Intr    85805    85901        +        0
     Intr    85988    86088        +        0
     Intr    86193    86356        +        0
     Intr    86443    86514        +        0
     Term    86712    86828        +        0

>4165340        /17788
```

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```
      len =      397    nex =        1

       Sngl   97179    97575          +         0

   >4185120         /6390

      len =     3432    nex =       13

       Term   15226    14939          -         0
       Intr   15428    15316          -         0
       Intr   15588    15539          -         0
       Intr   15779    15709          -         0
       Intr   15948    15879          -         0
       Intr   16280    16183          -         0
       Intr   16473    16407          -         0
       Intr   16656    16579          -         0
       Intr   16926    16759          -         0
       Intr   17196    17029          -         0
       Intr   17587    17291          -         0
       Intr   17910    17665          -         0
       Init   18370    18221          -         0

   >4185120         /96856

      len =      646    nex =        2

       Term   42352    41925          -         0
       Init   42557    42437          -         0

   >4185120         /25812

      len =      761    nex =        1

       Sngl   99742    98982          -         0

   >4185128         /92525

      len =     1425    nex =        2

       Init   13244    13883          +         0
       Term   14344    14668          +         0

   >4185128         /16416

      len =     1248    nex =        1

       Sngl   17454    18701          +         0

   >4185128         /17464

      len =     1840    nex =        5

       Term   32604    32490          -         0
       Intr   32905    32692          -         0
       Intr   33672    32987          -         0
       Intr   33912    33755          -         0
       Init   34329    34123          -         0

   >4185128         /26663
```

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```
len =    1076   nex =       4

 Init   38740   39208        +        0
 Intr   39413   39478        +        0
 Intr   39529   39651        +        0
 Term   39743   39815        +        0

>4185128        /24619

len =    1711   nex =       5

 Init   43094   43248        +        0
 Intr   43475   43537        +        0
 Intr   44259   44337        +        0
 Intr   44425   44470        +        0
 Term   44574   44804        +        0

>4185128        /14084

len =    1815   nex =       6

 Init   54517   54995        +        0
 Intr   55165   55247        +        0
 Intr   55403   55492        +        0
 Intr   55577   55725        +        0
 Intr   55817   55948        +        0
 Term   56044   56331        +        0

>4185128        /4231

len =    1128   nex =       1

 Sngl   57987   56860        -        0

>4185128        /37734

len =    1154   nex =       1

 Sngl    7894    6748        -        0

>4191760        /35366

len =    2418   nex =       7

 Term   51175   50955        -        0
 Intr   51401   51262        -        0
 Intr   51669   51495        -        0
 Intr   51922   51764        -        0
 Intr   52401   52256        -        0
 Intr   52598   52509        -        0
 Init   53372   52795        -        0

>4191760        /16162

len =    3435   nex =       7

 Term   54020   53640        -        0
 Intr   54593   54540        -        0
 Intr   54849   54707        -        0
 Intr   55352   54943        -        0
```

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```
         Intr    55948    55819          -          0
         Intr    56284    56184          -          0
         Init    57074    56736          -          0

>4191760        /27782

       len =     2185    nex =        6

         Term    68405    68073          -          0
         Intr    68552    68485          -          0
         Intr    68822    68687          -          0
         Intr    69025    68922          -          0
         Intr    69410    69139          -          0
         Init    70257    69851          -          0

>4191760        /31714

       len =      550    nex =        2

         Init    70697    70805          +          0
         Term    71007    71062          +          0

>4191771        /2312

       len =     2918    nex =       14

         Term    28814    28356          -          0
         Intr    28989    28901          -          0
         Intr    29148    29085          -          0
         Intr    29326    29244          -          0
         Intr    29548    29422          -          0
         Intr    29742    29658          -          0
         Intr    29885    29822          -          0
         Intr    30040    29979          -          0
         Intr    30224    30154          -          0
         Intr    30405    30310          -          0
         Intr    30627    30479          -          0
         Intr    30777    30708          -          0
         Intr    30887    30847          -          0
         Init    31273    30959          -          0

>4191771        /14853

       len =     2857    nex =       12

         Init    33231    33385          +          0
         Intr    33490    33533          +          0
         Intr    33762    33857          +          0
         Intr    33987    34079          +          0
         Intr    34302    34389          +          0
         Intr    34494    34555          +          0
         Intr    34638    34822          +          0
         Intr    34906    35083          +          0
         Intr    35171    35214          +          0
         Intr    35298    35514          +          0
         Intr    35611    35628          +          0
         Term    35711    36087          +          0

>4191771        /35176
```

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```
len =     2743   nex =       11

 Init   33285    33385        +         0
 Intr   33490    33533        +         0
 Intr   33762    33857        +         0
 Intr   33987    34079        +         0
 Intr   34302    34389        +         0
 Intr   34494    34555        +         0
 Intr   34638    34822        +         0
 Intr   34906    35083        +         0
 Intr   35171    35214        +         0
 Intr   35298    35514        +         0
 Term   35711    36017        +         0

>4191771        /33083

len =      217   nex =        1

 Sngl   54044    54260        +         0

>4191771        /125285

len =      447   nex =        1

 Sngl   65686    65240        -         0

>4191771        /152285

len =     1045   nex =        1

 Sngl   66311    65267        -         0

>4191771        /19555

len =     1320   nex =        3

 Term   71563    70726        -         0
 Intr   71884    71664        -         0
 Init   72045    71968        -         0

>4191771        /26452

len =      850   nex =        1

 Sngl   83459    83331        -         0

>4199934        /29227

len =     1035   nex =        2

 Init   19693    19801        +         0
 Term   20093    20727        +         0

>4199934        /33136

len =      338   nex =        1

 Sngl   20399    20729        +         0

>4199934        /154322
```

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```
     len =      352    nex =        1

      Sngl   23117   23462         +         0

>4199934        /36784

     len =     1133    nex =        1

      Sngl   23117   24249         +         0

>4204173        /41722

     len =     1054    nex =        2

      Term   21811   21377         -         0
      Init   22430   21898         -         0

>4204173        /14471

     len =     1120    nex =        3

      Term   23568   23254         -         0
      Intr   23859   23729         -         0
      Init   24373   23953         -         0

>4217996        /8441

     len =     1482    nex =        1

      Sngl   51080   52561         +         0

>4217996        /38968

     len =     2388    nex =        9

      Init   52825   53030         +         0
      Intr   53128   53176         +         0
      Intr   53267   53355         +         0
      Intr   53445   53501         +         0
      Intr   53636   53698         +         0
      Intr   53788   53853         +         0
      Intr   53992   54078         +         0
      Intr   54220   54313         +         0
      Term   54408   55212         +         0

>4218109        /324

     len =     1860    nex =        5

      Term   39406   38929         -         0
      Intr   39731   39476         -         0
      Intr   40077   39838         -         0
      Intr   40403   40276         -         0
      Init   40788   40484         -         0

>4218109        /1968

     len =     1690    nex =        5
```

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```
     Init   42662   43143        +        0
     Intr   43303   43336        +        0
     Intr   43644   43748        +        0
     Intr   43827   44015        +        0
     Term   44115   44344        +        0

>4218109        /36080

   len =     1657   nex =        4

     Term   53929   53688        -        0
     Intr   54126   54076        -        0
     Intr   54789   54211        -        0
     Init   55344   55008        -        0

>4218109        /38545

   len =      654   nex =        2

     Term   89772   89411        -        0
     Init   90064   89883        -        0

>4220468        /41875

   len =     1728   nex =        5

     Term   25333   24750        -        0
     Intr   25490   25414        -        0
     Intr   25978   25767        -        0
     Intr   26212   26057        -        0
     Init   26477   26297        -        0

>4220468        /917

   len =      599   nex =        1

     Sngl   26654   27252        +        0

>4220468        /25454

   len =      310   nex =        1

     Sngl   42788   42485        -        0

>4220468        /19157

   len =     1214   nex =        1

     Sngl   43720   42507        -        0

>4220468        /39244

   len =     2268   nex =        0

>4220468        /8247

   len =     1486   nex =        5

     Init   61354   61713        +        0
     Intr   61815   61920        +        0
     Intr   62016   62099       +      0
     Intr   62194   62322       +      0
     Term   62414   62839       +      0

>4220468        /9469

    len =     1120   nex =       5

     Term   75604   75323       -      0
     Intr   75828   75680       -      0
     Intr   76086   76037       -      0
     Intr   76234   76176       -      0
     Init   76442   76342       -      0

>4220468        /104853

    len =     1161   nex =       3

     Term   80528   80150       -      0
     Intr   80846   80610       -      0
     Init   81310   80910       -      0

>4220510        /33382

    len =     1810   nex =       2

     Term  119483  118560       -      0
     Init  120362  119827       -      0

>4220510        /39461

    len =     1133   nex =       2

     Term   62775   62278       -      0
     Init   63410   63166       -      0

>4220510        /36681

    len =     2001   nex =       3

     Init    8869    9090       +      0
     Intr    9370   10119       +      0
     Term   10211   10869       +      0

>4220627        /11357

    len =      670   nex =       2

     Term   23338   22980       -      0
     Init   23642   23419       -      0

>4220627        /1267

    len =     1798   nex =       2

     Init   30233   30321       +      0
     Term   30435   32030       +      0

>4220630        /10008
```

```
    len =     2590   nex =        9

     Term   16866   16556         -        0
     Intr   17015   16956         -        0
     Intr   17288   17193         -        0
     Intr   18114   18032         -        0
     Intr   18317   18255         -        0
     Intr   18553   18457         -        0
     Intr   18722   18641         -        0
     Intr   18974   18880         -        0
     Init   19138   19050         -        0

>4220630        /33813

    len =     1114   nex =        2

     Term    3819    3692         -        0
     Init    4805    4216         -        0

>4220631        /36799

    len =     2843   nex =       10

     Init   27324   27510         +        0
     Intr   27591   27698         +        0
     Intr   27785   27877         +        0
     Intr   27952   28044         +        0
     Intr   28143   28228         +        0
     Intr   28314   28592         +        0
     Intr   28670   28803         +        0
     Intr   28922   29014         +        0
     Intr   29085   29202         +        0
     Term   29308   29650         +        0

>4220631        /698

    len =      550   nex =        1

     Sngl   29083   29202         +        0

>4220631        /21717

    len =     3328   nex =        8

     Init   32801   32924         +        0
     Intr   33757   33803         +        0
     Intr   34429   34757         +        0
     Intr   34840   34922         +        0
     Intr   35182   35257         +        0
     Intr   35373   35437         +        0
     Intr   35509   35604         +        0
     Term   35716   36125         +        0

>4220631        /106086

    len =      430   nex =        2

     Init   43315   43344         +        0
     Term   43425   43740         +        0
```

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```
>4220631       /4634

  len =     271    nex =       1

   Sngl   43471   43741         +        0

>4220631       /21618

  len =    1544    nex =       1

   Sngl   52445   52155         -        0

>4220632       /21608

  len =    2002    nex =       6

   Init   15759   16019         +        0
   Intr   16186   16243         +        0
   Intr   16326   16397         +        0
   Intr   16993   17112         +        0
   Intr   17201   17302         +        0
   Term   17512   17760         +        0

>4220632       /33442

  len =     323    nex =       1

   Sngl   38225   38547         +        0

>4220632       /11562

  len =    1630    nex =       1

   Sngl   40558   40363         -        0

>4220632       /31276

  len =     539    nex =       2

   Term   42146   41798         -        0
   Init   42336   42218         -        0

>4220633       /6602

  len =    1537    nex =       2

   Init   24986   25221         +        0
   Term   25635   26107         +        0

>4220633       /14561

  len =    1210    nex =       1

   Sngl   41689   40482         -        0

>4220633       /43004

  len =     825    nex =       1

   Sngl   43944   44768         +        0
```

```
>4220633       /36013

  len =    2510   nex =      11

   Init   47139   47255       +         0
   Intr   47335   47437       +         0
   Intr   47521   47586       +         0
   Intr   47676   47799       +         0
   Intr   47876   48010       +         0
   Intr   48100   48160       +         0
   Intr   48246   48322       +         0
   Intr   48458   48613       +         0
   Intr   48705   48833       +         0
   Intr   48914   49046       +         0
   Term   49196   49300       +         0

>4220635       /11339

  len =    2031   nex =       7

   Init   38166   38351       +         0
   Intr   38520   38541       +         0
   Intr   38891   39017       +         0
   Intr   39176   39266       +         0
   Intr   39361   39450       +         0
   Intr   39711   39811       +         0
   Term   39886   40196       +         0

>4220635       /144690

  len =    2028   nex =       7

   Init   38172   38351       +         0
   Intr   38520   38541       +         0
   Intr   38891   39017       +         0
   Intr   39176   39266       +         0
   Intr   39361   39450       +         0
   Intr   39711   39811       +         0
   Term   39886   40199       +         0

>4220635       /7103

  len =     626   nex =       1

   Sngl   40942   41567       +         0

>4220635       /25758

  len =    1948   nex =       6

   Init   44440   44586       +         0
   Intr   44775   44978       +         0
   Intr   45285   45530       +         0
   Intr   45644   45886       +         0
   Intr   45969   46034       +         0
   Term   46128   46387       +         0

>4220635       /40185
```

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```
        len =     1376    nex =        1

         Sngl   53066   54441          +        0

    >4220636        /26820

        len =     2132    nex =       10

         Init   22952   23071          +        0
         Intr   23266   23317          +        0
         Intr   23412   23607          +        0
         Intr   23692   23771          +        0
         Intr   23869   23985          +        0
         Intr   24090   24140          +        0
         Intr   24233   24299          +        0
         Intr   24382   24485          +        0
         Intr   24578   24682          +        0
         Term   24783   25083          +        0

    >4220636        /121993

        len =     1306    nex =        5

         Term   37609   37394          -        0
         Intr   38097   37965          -        0
         Intr   38318   38196          -        0
         Intr   38425   38399          -        0
         Init   38699   38603          -        0

    >4220637        /29678

        len =     1417    nex =        2

         Term   13304   12526          -        0
         Init   13942   13414          -        0

    >4220637        /19279

        len =      614    nex =        1

         Sngl   17264   17877          +        0

    >4220637        /26411

        len =     1523    nex =        4

         Init   36910   37153          +        0
         Intr   37747   37816          +        0
         Intr   37913   38021          +        0
         Term   38124   38432          +        0

    >4220637        /5961

        len =     1390    nex =        4

         Init   38657   38747          +        0
         Intr   39193   39308          +        0
         Intr   39403   39466          +        0
         Term   39552   40045          +        0
```

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```
>4220637        /32574

    len =     2029    nex =        4

     Init    52862    53196         +         0
     Intr    53299    53516         +         0
     Intr    53613    53860         +         0
     Term    53997    54890         +         0

>4220638        /36765

    len =     1180    nex =        3

     Term    10773    10592         -         0
     Intr    11443    10925         -         0
     Init    11771    11524         -         0

>4220638        /16377

    len =     1782    nex =        4

     Term    10773    10360         -         0
     Intr    11443    10925         -         0
     Intr    11826    11524         -         0
     Init    12141    11914         -         0

>4220638        /7015

    len =      586    nex =        2

     Init    30811    30933         +         0
     Term    31055    31396         +         0

>4220638        /19110

    len =     1340    nex =        2

     Term    33848    32699         -         0
     Init    34038    33944         -         0

>4220638        /102368

    len =      730    nex =        2

     Init    40029    40250         +         0
     Term    40282    40754         +         0

>4220640        /32066

    len =      875    nex =        2

     Term      778      350         -         0
     Init     1099     1015         -         0

>4220640        /101893

    len =      646    nex =        1

     Sngl     1489     2131         +         0
```

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```
>4220640        /6915

   len =    2050   nex =       6

    Init   21763   22063        +        0
    Intr   22330   22386        +        0
    Intr   22578   22783        +        0
    Intr   22894   23167        +        0
    Intr   23311   23382        +        0
    Term   23473   23804        +        0

>4220640        /28838

   len =     594   nex =       1

    Sngl   27690   27097        -        0

>4220640        /33726

   len =    1839   nex =       0

>4220640        /37012

   len =     441   nex =       1

    Sngl   31588   32028        +        0

>4220640        /20116

   len =    1516   nex =       4

    Term   32430   31988        -        0
    Intr   32676   32525        -        0
    Intr   33332   32969        -        0
    Init   33503   33404        -        0

>4220640        /6156

   len =    1498   nex =       3

    Term   32430   32015        -        0
    Intr   32676   32525        -        0
    Init   33512   32969        -        0

>4220640        /10926

   len =    1533   nex =       4

    Term   32430   32015        -        0
    Intr   32676   32525        -        0
    Intr   33332   32969        -        0
    Init   33547   33404        -        0

>4220640        /2767

   len =    1053   nex =       3

    Term   34226   34114        -        0
    Intr   34482   34391        -        0
    Init   34843   34602        -        0
```

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```
>4220640        /102455

    len =     1393    nex =       5

      Init    36025    36150          +        0
      Intr    36243    36283          +        0
      Intr    36497    36565          +        0
      Intr    36651    36707          +        0
      Term    36797    37018          +        0

>4220640        /6800

    len =     1697    nex =       5

      Term    45977    45853          -        0
      Intr    46279    46055          -        0
      Intr    46510    46382          -        0
      Intr    46828    46790          -        0
      Init    47542    47110          -        0

>4220640        /141813

    len =      951    nex =       1

      Sngl     5195     4245          -        0

>4220640        /40646

    len =     3764    nex =       4

      Init    65692    65939          +        0
      Intr    66421    66558          +        0
      Intr    66651    66740          +        0
      Term    66834    66890          +        0

>4220640        /28790

    len =     1814    nex =       8

      Init    71705    71840          +        0
      Intr    71931    71971          +        0
      Intr    72059    72313          +        0
      Intr    72390    72450          +        0
      Intr    72546    72670          +        0
      Intr    72752    72814          +        0
      Intr    72899    73023          +        0
      Term    73185    73518          +        0

>4220640        /10103

    len =      610    nex =       1

      Sngl    82414    83015          +        0

>4220641        /41308

    len =     1514    nex =       6

      Term    16718    16586          -        0
```

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```
        Intr   16848    16803          -         0
        Intr   17051    16951          -         0
        Intr   17286    17149          -         0
        Intr   17585    17487          -         0
        Init   17849    17733          -         0

>4220641         /11265

      len =     1899    nex =         6

        Term   28923    28663          -         0
        Intr   29185    29124          -         0
        Intr   29344    29270          -         0
        Intr   29971    29425          -         0
        Intr   30137    30073          -         0
        Init   30561    30314          -         0

>4220641         /1152

      len =     1189    nex =         3

        Term   68284    68140          -         0
        Intr   68713    68513          -         0
        Init   69328    69078          -         0

>4220643         /125902

      len =      550    nex =         1

        Sngl   11332    11876          +         0

>4220643         /8788

      len =     1231    nex =         3

        Term   12170    11960          -         0
        Intr   12444    12249          -         0
        Init   13190    12769          -         0

>4220643         /10876

      len =     1876    nex =         6

        Init   13443    13678          +         0
        Intr   13778    13880          +         0
        Intr   14150    14217          +         0
        Intr   14462    14653          +         0
        Intr   14738    14856          +         0
        Term   14959    15318          +         0

>4220643         /109639

      len =     2710    nex =         5

        Term   18433    18225          -         0
        Intr   20036    19936          -         0
        Intr   20200    20123          -         0
        Intr   20322    20279          -         0
        Init   20572    20403          -         0
```

```
>4220643        /92267

   len =     1549    nex =        6

    Term   27866   27561          -          0
    Intr   28045   27980          -          0
    Intr   28239   28143          -          0
    Intr   28564   28318          -          0
    Intr   28840   28748          -          0
    Init   29109   28937          -          0

>4220643        /2347

   len =      833    nex =        1

    Sngl   35179   34347          -          0

>4220643        /10875

   len =     2193    nex =        6

    Term    2953    2902          -          0
    Intr    3203    3096          -          0
    Intr    3434    3291          -          0
    Intr    3609    3517          -          0
    Intr    3792    3721          -          0
    Init    4031    3873          -          0

>4220643        /126592

   len =     2377    nex =        6

    Term    7320    7059          -          0
    Intr    7755    7637          -          0
    Intr    8012    7841          -          0
    Intr    8587    8399          -          0
    Intr    9007    8930          -          0
    Init    9247    9210          -          0

>4220644        /40534

   len =     2331    nex =        6

    Term   14665   14123          -          0
    Intr   15328   14749          -          0
    Intr   15498   15413          -          0
    Intr   15667   15597          -          0
    Intr   15888   15738          -          0
    Init   16453   16250          -          0

>4220644        /39041

   len =     1930    nex =        5

    Init   17880   18258          +          0
    Intr   18361   18441          +          0
    Intr   18898   19002          +          0
    Intr   19139   19288          +          0
    Term   19396   19805          +          0
```

```
>4220644        /12996

   len =     2758    nex =        8

    Init     3859     4029          +         0
    Intr     4630     4933          +         0
    Intr     5050     5142          +         0
    Intr     5216     5349          +         0
    Intr     5438     5564          +         0
    Intr     5941     6046          +         0
    Intr     6174     6245          +         0
    Term     6344     6616          +         0

>4220644        /40770

   len =     2268    nex =       10

    Term    60836    60743          -         0
    Intr    60995    60915          -         0
    Intr    61151    61082          -         0
    Intr    61384    61296          -         0
    Intr    61819    61711          -         0
    Intr    62039    61921          -         0
    Intr    62183    62124          -         0
    Intr    62462    62339          -         0
    Intr    62674    62574          -         0
    Init    63010    62903          -         0

>4220644        /37152

   len =     2246    nex =        5

    Init    68479    69417          +         0
    Intr    69503    69580          +         0
    Intr    69662    69773          +         0
    Intr    70259    70309          +         0
    Term    70434    70724          +         0

>4220645        /19689

   len =     1479    nex =        3

    Init    18327    18524          +         0
    Intr    18628    18878          +         0
    Term    19278    19805          +         0

>4220645        /102797

   len =     1492    nex =        0

>4220645        /40166

   len =      430    nex =        1

    Sngl    19392    19821          +         0

>4220645        /8735

   len =     1330    nex =        6
```

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```
     Term   20047   19864        -        0
     Intr   20212   20150        -        0
     Intr   20365   20303        -        0
     Intr   20513   20457        -        0
     Intr   20721   20611        -        0
     Init   21190   20957        -        0

>4220645        /29889

    len =     1879    nex =        3

     Init   24415   24465        +        0
     Intr   24561   25553        +        0
     Term   25635   26068        +        0

>4220645        /24775

    len =     1521    nex =        5

     Term    2820    2592        -        0
     Intr    3077    2961        -        0
     Intr    3417    3180        -        0
     Intr    3923    3810        -        0
     Init    4112    4012        -        0

>4220645        /8347

    len =      531    nex =        1

     Sngl   53094   52564        -        0

>4220645        /30517

    len =     1717    nex =        5

     Init   69636   70034        +        0
     Intr   70115   70245        +        0
     Intr   70339   70572        +        0
     Intr   70658   70913        +        0
     Term   71018   71352        +        0

>4220645        /13425

    len =      573    nex =        2

     Init   70771   70913        +        0
     Term   71018   71340        +        0

>4235150        /15739

    len =      894    nex =        1

     Sngl   33125   32244        -        0

>4235150        /21075

    len =      811    nex =        2

     Init   52126   52284        +        0
     Term   52598   52936        +        0
```

```
>4235150        /110090

    len =      778   nex =        2

     Init   52159   52284          +        0
     Term   52598   52936          +        0

>4235150        /30523

    len =      537   nex =        1

     Sngl   54031   54567          +        0

>4235150        /32316

    len =      521   nex =        1

     Sngl   56193   56713          +        0

>4235150        /111638

    len =     1210   nex =        3

     Term   71107   70335          -        0
     Intr   71256   71195          -        0
     Init   71543   71346          -        0

>4235150        /32272

    len =     1224   nex =        3

     Term   71107   70354          -        0
     Intr   71256   71195          -        0
     Init   71577   71346          -        0

>4235150        /25124

    len =     1278   nex =        3

     Term   71107   70354          -        0
     Intr   71256   71195          -        0
     Init   71631   71346          -        0

>4249393        /6819

    len =     1766   nex =        2

     Term   43545   42141          -        0
     Init   43906   43634          -        0

>4249393        /6017

    len =     2858   nex =       14

     Init   47446   47702          +        0
     Intr   47789   47851          +        0
     Intr   47928   48017          +        0
     Intr   48101   48160          +        0
     Intr   48246   48311          +        0
```

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```
     Intr   48386   48451        +        0
     Intr   48684   48748        +        0
     Intr   48827   48919        +        0
     Intr   49182   49266        +        0
     Intr   49347   49390        +        0
     Intr   49467   49529        +        0
     Intr   49655   49708        +        0
     Intr   49773   49832        +        0
     Term   49904   50303        +        0

>4249393       /8332

    len =     670   nex =        3

     Init   49619   49708        +        0
     Intr   49773   49832        +        0
     Term   49904   50282        +        0

>4249393       /38690

    len =    2474   nex =        4

     Term   51247   50779        -        0
     Intr   51460   51331        -        0
     Intr   51783   51576        -        0
     Init   53252   52424        -        0

>4249393       /34081

    len =    1122   nex =        4

     Init   65982   66175        +        0
     Intr   66322   66532        +        0
     Intr   66615   66784        +        0
     Term   66874   67103        +        0

>4249393       /39971

    len =     655   nex =        1

     Sngl   72891   72237        -        0

>4262221       /40457

    len =     532   nex =        1

     Sngl   10235   10766        +        0

>4262221       /19163

    len =    1709   nex =        2

     Term   17710   16611        -        0
     Init   18319   18101        -        0

>4262221       /17996

    len =    2650   nex =        2

     Init   24589   25919        +        0
```

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```
      Term   26031   27234         +        0

   >4262221       /94462

     len =     1422   nex =        6

      Term   42754   42345         -        0
      Intr   42956   42854         -        0
      Intr   43130   43051         -        0
      Intr   43426   43369         -        0
      Intr   43616   43519         -        0
      Init   43766   43687         -        0

   >4262221       /108290

     len =     1635   nex =        6

      Term   42754   42345         -        0
      Intr   42956   42854         -        0
      Intr   43130   43051         -        0
      Intr   43426   43369         -        0
      Intr   43616   43519         -        0
      Init   43869   43687         -        0

   >4262221       /8949

     len =     2493   nex =        6

      Term   45313   44902         -        0
      Intr   45683   45592         -        0
      Intr   45851   45766         -        0
      Intr   46447   46219         -        0
      Intr   46760   46534         -        0
      Init   47394   46849         -        0

   >4262221       /3831

     len =     1431   nex =        1

      Sngl   59938   58508         -        0

   >4262221       /120459

     len =      670   nex =        1

      Sngl   61325   60659         -        0

   >4262221       /22418

     len =     1779   nex =        4

      Term    5241    4407         -        0
      Intr    5454    5345         -        0
      Intr    5819    5550         -        0
      Init    6185    5932         -        0

   >4262221       /37962

     len =     1312   nex =        3
```

```
        Init   73550   73783        +        0
        Intr   73889   74305        +        0
        Term   74608   74861        +        0

>4262221         /2877

      len =    2110    nex =       7

        Term   75024   74744        -        0
        Intr   75175   75108        -        0
        Intr   75424   75248        -        0
        Intr   75663   75511        -        0
        Intr   75879   75796        -        0
        Intr   76233   76087        -        0
        Init   76452   76319        -        0

>4263038         /95374

      len =     430    nex =       1

        Sngl   43068   43493        +        0

>4263038         /3879

      len =     642    nex =       1

        Sngl   43783   43142        -        0

>4263373         /255

      len =    7618    nex =       4

        Term   38456   36387        -        0
        Intr   40079   40029        -        0
        Intr   43641   40959        -        0
        Init   44004   43817        -        0

>4263373         /4059

      len =    7616    nex =       4

        Term   38456   36389        -        0
        Intr   40079   40029        -        0
        Intr   43641   40959        -        0
        Init   44004   43817        -        0

>4263373         /42686

      len =    1090    nex =       2

        Term   58099   57610        -        0
        Init   58697   58409        -        0

>4263373         /28019

      len =    1179    nex =       2

        Term   58099   57546        -        0
        Init   58724   58409        -        0
```

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```
>4263586        /2771

     len =      850    nex =        5

      Init   44628    44729         +        0
      Intr   44822    44985         +        0
      Intr   45066    45099         +        0
      Intr   45171    45264         +        0
      Term   45352    45476         +        0

>4263586        /42384

     len =      868    nex =        5

      Init   44631    44729         +        0
      Intr   44822    44985         +        0
      Intr   45066    45099         +        0
      Intr   45171    45264         +        0
      Term   45352    45498         +        0

>4263586        /34420

     len =     1907    nex =        8

      Term   54050    53865         -        0
      Intr   54344    54138         -        0
      Intr   54536    54429         -        0
      Intr   54749    54633         -        0
      Intr   54915    54829         -        0
      Intr   55154    54990         -        0
      Intr   55337    55226         -        0
      Init   55771    55407         -        0

>4263586        /1237

     len =     2153    nex =        8

      Init   55911    56299         +        0
      Intr   56483    56550         +        0
      Intr   56661    56794         +        0
      Intr   56893    56981         +        0
      Intr   57082    57139         +        0
      Intr   57205    57257         +        0
      Intr   57350    57562         +        0
      Term   57841    58063         +        0

>4263694        /19875

     len =      171    nex =        1

      Sngl   10500    10333         -        0

>4263694        /31665

     len =      733    nex =        3

      Term   10901    10692         -        0
      Intr   11155    11017         -        0
      Init   11424    11303         -        0
```

```
>4263694        /40344

   len =      701    nex =        2

    Term   14673   14535          -          0
    Init   14888   14792          -          0

>4263694        /159279

   len =      746    nex =        1

    Sngl   16225   16970          +          0

>4263694        /32907

   len =      790    nex =        3

    Term   29067   28667          -          0
    Intr   29226   29161          -          0
    Init   29452   29310          -          0

>4263694        /37176

   len =     1706    nex =        7

    Term   29067   28646          -          0
    Intr   29226   29161          -          0
    Intr   29522   29310          -          0
    Intr   29704   29617          -          0
    Intr   29841   29804          -          0
    Intr   30029   29932          -          0
    Init   30183   30118          -          0

>4263694        /35952

   len =     1351    nex =        4

    Term   43833   43628          -          0
    Intr   44147   44072          -          0
    Intr   44489   44259          -          0
    Init   44978   44842          -          0

>4263694        /13834

   len =     1330    nex =        5

    Init   49040   49096          +          0
    Intr   49251   49313          +          0
    Intr   49548   49848          +          0
    Intr   49937   50002          +          0
    Term   50123   50363          +          0

>4263694        /13453

   len =     1270    nex =        4

    Init   49251   49313          +          0
    Intr   49548   49848          +          0
    Intr   49937   50002          +          0
    Term   50123   50363          +          0
```

```
>4263694      /9338

   len =     754   nex =       3

    Init   49575   49848         +        0
    Intr   49937   50002         +        0
    Term   50123   50328         +        0

>4263694       /15582

   len =    2721   nex =       9

    Term   52117   51580         -        0
    Intr   52340   52248         -        0
    Intr   52602   52434         -        0
    Intr   52976   52716         -        0
    Intr   53226   53078         -        0
    Intr   53488   53326         -        0
    Intr   53624   53560         -        0
    Intr   53954   53704         -        0
    Init   54300   54063         -        0

>4263694       /21201

   len =     707   nex =       1

    Sngl   57642   58348         +        0

>4263694       /35447

   len =     804   nex =       2

    Init   79508   79806         +        0
    Term   79888   80311         +        0

>4263694       /37871

   len =     460   nex =       2

    Init   79509   79806         +        0
    Term   79888   79968         +        0

>4263753       /29201

   len =    1898   nex =       5

    Term   23901   23530         -        0
    Intr   24129   24008         -        0
    Intr   24602   24242         -        0
    Intr   25041   24923         -        0
    Init   25424   25374         -        0

>4263753       /38824

   len =    2017   nex =       5

    Term   23901   23444         -        0
    Intr   24129   24008         -        0
    Intr   24602   24242         -        0
```

09955325 .061501

```
     Intr   25041   24923          -         0
     Init   25447   25374          -         0

>4263753        /32341

   len =     1853   nex =        4

     Term   25964   25590          -         0
     Intr   26181   26057          -         0
     Intr   26645   26279          -         0
     Init   27442   27181          -         0

>4263753        /18342

   len =     1999   nex =        3

     Term   25964   25587          -         0
     Intr   26181   26057          -         0
     Init   26645   26279          -         0

>4263753        /142593

   len =     2369   nex =        9

     Term   27931   27684          -         0
     Intr   28203   28139          -         0
     Intr   28363   28298          -         0
     Intr   28872   28453          -         0
     Intr   29202   29080          -         0
     Intr   29421   29303          -         0
     Intr   29591   29498          -         0
     Intr   29825   29715          -         0
     Init   30052   29909          -         0

>4263753        /114575

   len =     2332   nex =        3

     Term   40874   40258          -         0
     Intr   42140   40970          -         0
     Init   42589   42333          -         0

>4263753        /104891

   len =     1090   nex =        1

     Sngl   49712   49632          -         0

>4263753        /34854

   len =      771   nex =        0

>4263753        /7959

   len =     1461   nex =        1

     Sngl   49712   49320          -         0

>4263753        /22013
```

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```
     len =     1473    nex =        3

       Init   59531    59715          +        0
       Intr   60399    60450          +        0
       Term   60528    61003          +        0

  >4263753         /121365

     len =     1415    nex =        2

       Init   59581    59715          +        0
       Term   60399    60450          +        0

  >4263753         /8599

     len =     1015    nex =        1

       Sngl   72614    72191          -        0

  >4263753         /42931

     len =     2493    nex =        4

       Term   72614    72054          -        0
       Intr   73868    73162          -        0
       Intr   74336    74237          -        0
       Init   74546    74430          -        0

  >4263753         /33691

     len =     2530    nex =        4

       Term   72614    72054          -        0
       Intr   73868    73162          -        0
       Intr   74336    74237          -        0
       Init   74574    74430          -        0

  >4263762         /24286

     len =     2117    nex =        3

       Init   71346    71674          +        0
       Intr   71760    71795          +        0
       Term   73186    73462          +        0

  >4263774         /9149

     len =     1339    nex =        3

       Term   11294    10832          -        0
       Intr   11773    11641          -        0
       Init   12170    11858          -        0

  >4263774         /8134

     len =     1118    nex =        2

       Term     959      793          -        0
       Init    1290     1112          -        0
```

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```
>4263774        /112110

  len =    1307   nex =        2

   Term     959     618         -        0
   Init    1290    1112         -        0

>4263774        /5598

  len =    1055   nex =        2

   Init   22583   22724         +        0
   Term   23358   23637         +        0

>4263774        /9996

  len =     571   nex =        1

   Sngl   29990   30560         +        0

>4263774        /22447

  len =    1408   nex =        2

   Term    2804    2016         -        0
   Init    3423    3330         -        0

>4263774        /40628

  len =    2445   nex =        9

   Init    4021    4390         +        0
   Intr    4472    4567         +        0
   Intr    4643    4735         +        0
   Intr    4808    4881         +        0
   Intr    4958    5224         +        0
   Intr    5677    5804         +        0
   Intr    5890    5985         +        0
   Intr    6073    6178         +        0
   Term    6264    6465         +        0

>4263774        /38267

  len =    2290   nex =        6

   Init   45101   45783         +        0
   Intr   45981   46103         +        0
   Intr   46189   46282         +        0
   Intr   46402   46484         +        0
   Intr   46569   46658         +        0
   Term   46747   47387         +        0

>4263774        /2403

  len =    1055   nex =        3

   Term   62305   62089         -        0
   Intr   62745   62486         -        0
   Init   63143   63001         -        0
```

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```
>4309719        /38985

   len =     748    nex =        2

    Term   21312   20917         -          0
    Init   21664   21527         -          0

>4309719        /692

   len =    1100    nex =        2

    Init   24887   24997         +          0
    Term   25486   25986         +          0

>4309719        /5695

   len =    1349    nex =        5

    Term   26228   25952         -          0
    Intr   26479   26399         -          0
    Intr   26755   26593         -          0
    Intr   26979   26869         -          0
    Init   27300   27066         -          0

>4309719        /105626

   len =    1111    nex =        2

    Term   33999   33726         -          0
    Init   34836   34438         -          0

>4309719        /4845

   len =    1063    nex =        1

    Sngl   43384   44446         +          0

>4309719        /2731

   len =     971    nex =        3

    Init   60881   61402         +          0
    Intr   61481   61699         +          0
    Term   61778   61851         +          0

>4309719        /32047

   len =    1818    nex =        6

    Init   65313   65489         +          0
    Intr   65653   65708         +          0
    Intr   65790   65882         +          0
    Intr   65980   66050         +          0
    Intr   66683   66787         +          0
    Term   66874   67130         +          0

>4309719        /8068

   len =    2380    nex =        8
```

09595326 .061600

```
        Init   81136   81254        +       0
        Intr   82137   82165        +       0
        Intr   82264   82329        +       0
        Intr   82552   82659        +       0
        Intr   82754   82819        +       0
        Intr   82909   83029        +       0
        Intr   83121   83202        +       0
        Term   83284   83515        +       0

>4309719       /150251

      len =     550    nex =      2

        Init   83121   83202        +       0
        Term   83284   83538        +       0

>4309719       /35743

      len =    1049    nex =      1

        Sngl   89585   90633        +       0

>4309747       /34060

      len =    1132    nex =      5

        Init   38591   38699        +       0
        Intr   38809   38862        +       0
        Intr   39074   39183        +       0
        Intr   39346   39430        +       0
        Term   39528   39722        +       0

>4314354       /1650

      len =    1096    nex =      2

        Init   33624   33916        +       0
        Term   34315   34719        +       0

>4314354       /19093

      len =    1161    nex =      5

        Term   36756   36705        -       0
        Intr   36980   36836        -       0
        Intr   37153   37045        -       0
        Intr   37293   37236        -       0
        Init   37466   37365        -       0

>4314354       /107116

      len =     379    nex =      1

        Sngl   41136   41514        +       0

>4314354       /37274

      len =    2230    nex =      6

        Init   46941   47138        +       0
```

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```
    Intr   47607   48124        +       0
    Intr   48222   48451        +       0
    Intr   48548   48689        +       0
    Intr   48776   48837        +       0
    Term   48917   49168        +       0

>4314354       /4014

   len =    1725   nex =       1

    Sngl   61481   60193        -       0

>4314374       /18569

   len =    1010   nex =       3

    Term     443      32        -       0
    Intr     853     747        -       0
    Init    1041    1020        -       0

>4314374       /38329

   len =    2442   nex =       8

    Init    2646    2694        +       0
    Intr    2782    2852        +       0
    Intr    3021    3085        +       0
    Intr    3181    3225        +       0
    Intr    3316    3396        +       0
    Intr    3473    3540        +       0
    Intr    3616    3664        +       0
    Term    3771    3925        +       0

>4314374       /35081

   len =     839   nex =       2

    Term   22049   21488        -       0
    Init   22326   22150        -       0

>4314374       /20875

   len =    2177   nex =       9

    Term   42829   42511        -       0
    Intr   43040   42944        -       0
    Intr   43426   43260        -       0
    Intr   43549   43516        -       0
    Intr   43684   43638        -       0
    Intr   43912   43761        -       0
    Intr   44106   44015        -       0
    Intr   44262   44214        -       0
    Init   44687   44480        -       0

>4314374       /1126

   len =    1671   nex =       3

    Term   57444   57236        -       0
    Intr   58681   57651        -       0
```

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```
     Init   58906   58778          -       0

>4314374        /26333

    len =    1393   nex =        0

>4325340        /27144

    len =     460   nex =        1

     Sngl     950     630          -       0

>4325340        /19541

    len =     670   nex =        1

     Sngl     950     636          -       0

>4325340        /122423

    len =     624   nex =        1

     Sngl   30231   30854          +       0

>4325352        /9742

    len =     131   nex =        1

     Sngl   51030   51160          +       0

>4325365        /33985

    len =    2158   nex =        6

     Term     346       1          -       0
     Intr     977     867          -       0
     Intr    1268    1059          -       0
     Intr    1516    1383          -       0
     Intr    1864    1705          -       0
     Init    2158    1967          -       0

>4325365        /35963

    len =     946   nex =        2

     Init   65779   66009          +       0
     Term   66230   66707          +       0

>4325365        /3552

    len =    1150   nex =        1

     Sngl   93731   94876          +       0

>4325365        /6659

    len =    1125   nex =        1

     Sngl   93738   94862          +       0
```

```
>4325365        /36687

   len =     1990    nex =        1

     Sngl   99721  100926          +        0

>4335711        /5

   len =     1270    nex =        1

     Sngl  115475  114208          -        0

>4335711        /9064

   len =      650    nex =        0

>4335711        /7216

   len =     1048    nex =        1

     Sngl   35552   34505          -        0

>4335711        /7792

   len =     1193    nex =        3

     Init   46238   46470          +        0
     Intr   46624   46739          +        0
     Term   47125   47430          +        0

>4335711        /122361

   len =      718    nex =        2

     Init   46239   46470          +        0
     Term   46624   46956          +        0

>4335711        /7201

   len =     1192    nex =        3

     Init   46239   46470          +        0
     Intr   46624   46739          +        0
     Term   47125   47430          +        0

>4335711        /40916

   len =     1194    nex =        3

     Init   46239   46470          +        0
     Intr   46624   46739          +        0
     Term   47125   47432          +        0

>4335711        /9723

   len =     2068    nex =        1

     Sngl   82273   80206          -        0

>4335744        /41585
```

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```
     len =     2418    nex =        9

      Term   10913    10690          -         0
      Intr   11117    11009          -         0
      Intr   11446    11232          -         0
      Intr   11721    11575          -         0
      Intr   12110    11952          -         0
      Intr   12261    12202          -         0
      Intr   12404    12337          -         0
      Intr   12578    12482          -         0
      Init   13107    12916          -         0

>4335744        /35226

     len =      900    nex =        1

      Sngl   39634    40533          +         0

>4335744        /41376

     len =     2132    nex =        8

      Init   47695    47718          +         0
      Intr   47825    47884          +         0
      Intr   48035    48129          +         0
      Intr   48223    48395          +         0
      Intr   48485    48597          +         0
      Intr   48707    48752          +         0
      Intr   48859    48899          +         0
      Term   48974    49259          +         0

>4335744        /1697

     len =     1601    nex =        3

      Init   58565    58779          +         0
      Intr   59480    59534          +         0
      Term   59634    60165          +         0

>4337186        /33524

     len =      173    nex =        1

      Sngl    2653     2825          +         0

>4337186        /141837

     len =      392    nex =        1

      Sngl   28822    28431          -         0

>4337186        /110587

     len =      720    nex =        1

      Sngl   39509    39644          +         0

>4337186        /16295
```

09595326.061600

```
   len =    3190   nex =      11

     Init   44064   44289         +        0
     Intr   44410   44537         +        0
     Intr   44623   44694         +        0
     Intr   44771   44884         +        0
     Intr   45009   45077         +        0
     Intr   45167   45277         +        0
     Intr   45503   45565         +        0
     Intr   45889   46044         +        0
     Intr   46155   46235         +        0
     Intr   46520   46648         +        0
     Term   46942   47253         +        0

>4337186        /7536

   len =    2080   nex =      10

     Term   62706   62383         -        0
     Intr   62883   62815         -        0
     Intr   63047   62962         -        0
     Intr   63232   63146         -        0
     Intr   63400   63322         -        0
     Intr   63600   63523         -        0
     Intr   63730   63689         -        0
     Intr   63936   63836         -        0
     Intr   64142   64032         -        0
     Init   64462   64286         -        0

>4337186        /6917

   len =     277   nex =       1

     Sngl     766    1042         +        0

>4371278        /15139

   len =    1126   nex =       3

     Init   15864   16132         +        0
     Intr   16428   16560         +        0
     Term   16673   16989         +        0

>4371278        /23523

   len =     679   nex =       1

     Sngl   18840   19518         +        0

>4371278        /108612

   len =     457   nex =       1

     Sngl   18849   19305         +        0

>4371278        /11998

   len =    1361   nex =       4

     Term   20141   19887         -        0
```

```
        Intr   20371   20231         -        0
        Intr   20758   20463         -        0
        Init   21247   20880         -        0

>4371278         /25220

      len =     1413   nex =        4

        Init   24105   24468         +        0
        Intr   24590   24885         +        0
        Intr   24977   25117         +        0
        Term   25216   25517         +        0

>4371278         /38965

      len =     1137   nex =        4

        Init   24195   24468         +        0
        Intr   24590   24885         +        0
        Intr   24977   25117         +        0
        Term   25216   25331         +        0

>4371278         /3571

      len =     1941   nex =        5

        Init   38901   39030         +        0
        Intr   39208   39356         +        0
        Intr   39462   39650         +        0
        Intr   39739   39907         +        0
        Term   40334   40841         +        0

>4371278         /23542

      len =      381   nex =        1

        Sngl    4113    4343         +        0

>4371278         /1517

      len =      850   nex =        2

        Init   43998   44170         +        0
        Term   44506   44846         +        0

>4371278         /2454

      len =     1371   nex =        3

        Term   45469   45000         -        0
        Intr   45776   45574         -        0
        Init   46370   45982         -        0

>4371278         /38677

      len =     1210   nex =        1

        Sngl   46951   48155         +        0

>4376087         /92448
```

```
    len =     708   nex =       2

      Init  100575  100763        +        0
      Term  100977  101282        +        0

>4376087        /30438

    len =    2020   nex =       6

      Init  100575  100763        +        0
      Intr  100977  101158        +        0
      Intr  101420  101606        +        0
      Intr  101697  101858        +        0
      Intr  101967  102269        +        0
      Term  102338  102594        +        0

>4376087        /18909

    len =    2031   nex =       9

      Term  110792  110506        -        0
      Intr  110969  110872        -        0
      Intr  111157  111078        -        0
      Intr  111325  111254        -        0
      Intr  111459  111418        -        0
      Intr  111712  111551        -        0
      Intr  112024  111796        -        0
      Intr  112222  112153        -        0
      Init  112536  112404        -        0

>4376087        /26540

    len =    1176   nex =       6

      Term  122365  122161        -        0
      Intr  122548  122446        -        0
      Intr  122806  122654        -        0
      Intr  122940  122913        -        0
      Intr  123065  123029        -        0
      Init  123336  123143        -        0

>4376087        /18266

    len =    1184   nex =       2

      Term  124539  123889        -        0
      Init  125072  124716        -        0

>4376087        /1794

    len =     835   nex =       3

      Term   12383   12097        -        0
      Intr   12576   12475        -        0
      Init   12931   12658        -        0

>4376087        /11274

    len =    1278   nex =       5
```

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```
  Init  130626  130868         +        0
  Intr  130994  131032         +        0
  Intr  131111  131263         +        0
  Intr  131398  131473         +        0
  Term  131661  131903         +        0

>4376087       /13475

len =    2498   nex =        8

  Init  174373  174669         +        0
  Intr  175057  175144         +        0
  Intr  175243  175307         +        0
  Intr  175412  175460         +        0
  Intr  175723  175775         +        0
  Intr  176218  176333         +        0
  Intr  176431  176488         +        0
  Term  176591  176870         +        0

>4376087       /19707

len =     799   nex =        1

  Sngl   26682   25892         -        0

>4376087       /27837

len =    1298   nex =        0

>4376087       /39370

len =    1124   nex =        1

  Sngl   27194   26071         -        0

>4376087       /16131

len =     691   nex =        1

  Sngl   43223   42533         -        0

>4376087       /108940

len =    1463   nex =        5

  Term   89305   88955         -        0
  Intr   89463   89386         -        0
  Intr   89742   89557         -        0
  Intr   90200   90148         -        0
  Init   90417   90284         -        0

>4388714       /40273

len =    3039   nex =        4

  Init   79641   79729         +        0
  Intr   79806   79946         +        0
  Intr   81346   81476         +        0
  Term   81558   82484         +        0
```

09855326.061502

```
>4388714        /39234

    len =    1951   nex =       3

     Init   79641   79729        +       0
     Intr   79806   79946        +       0
     Term   81346   81472        +       0

>4388714        /103540

    len =     477   nex =       1

     Sngl   82014   82484        +       0

>4406752        /2021

    len =    1990   nex =       5

     Init   16077   16503        +       0
     Intr   16583   16721        +       0
     Intr   16810   16963        +       0
     Intr   17036   17159        +       0
     Term   17250   17624        +       0

>4406752        /20712

    len =    1210   nex =       2

     Init   42530   42826        +       0
     Term   42920   43154        +       0

>4406752        /390

    len =     627   nex =       2

     Init   42528   42826        +       0
     Term   42920   43154        +       0

>4406752        /18785

    len =    1039   nex =       2

     Term   43763   43148        -       0
     Init   44186   43973        -       0

>4406752        /38210

    len =    1270   nex =       2

     Term   64451   63872        -       0
     Init   65132   64859        -       0

>4406752        /3964

    len =    1154   nex =       1

     Sngl   70202   69049        -       0

>4406752        /17089
```

09555325.061500

```
    len =    2174   nex =       9

     Init   77860   78033        +        0
     Intr   78111   78291        +        0
     Intr   78423   78565        +        0
     Intr   78666   78729        +        0
     Intr   78889   78941        +        0
     Intr   79177   79232        +        0
     Intr   79324   79398        +        0
     Intr   79482   79670        +        0
     Term   79755   80033        +        0

>4406776       /36238

    len =    2338   nex =       4

     Init   59059   59158        +        0
     Intr   59414   59731        +        0
     Intr   59810   60366        +        0
     Term   60442   61396        +        0

>4406776       /8529

    len =     528   nex =       1

     Sngl   60863   61390        +        0

>4406776       /39285

    len =    1992   nex =       0

>4406776       /33274

    len =     310   nex =       1

     Sngl   61563   61868        +        0

>4406790       /110175

    len =     473   nex =       1

     Sngl   27736   27628        -        0

>4406790       /14605

    len =    2614   nex =       9

     Init   72541   72737        +        0
     Intr   72828   72926        +        0
     Intr   73016   73126        +        0
     Intr   73401   73494        +        0
     Intr   73588   73688        +        0
     Intr   73852   73953        +        0
     Intr   74050   74163        +        0
     Intr   74379   74453        +        0
     Term   74542   74819        +        0

>4406790       /4489
```

```
    len =     396   nex =       1

     Sngl   81642   81247         -        0

>4406805        /11178

    len =     814   nex =       2

     Term   29635   29022         -        0
     Init   29835   29708         -        0

>4406805        /112970

    len =     816   nex =       2

     Term   29635   29024         -        0
     Init   29839   29708         -        0

>4406805        /42057

    len =     156   nex =       1

     Sngl   33661   33506         -        0

>4406805        /37664

    len =    1390   nex =       2

     Term   32476   32343         -        0
     Init   33725   33309         -        0

>4406805        /40712

    len =    1607   nex =       4

     Term   34590   34119         -        0
     Intr   34839   34695         -        0
     Intr   35037   34925         -        0
     Init   35725   35374         -        0

>4406805        /35337

    len =    1658   nex =       4

     Term    2559    2284         -        0
     Intr    2866    2649         -        0
     Intr    3066    2950         -        0
     Init    3279    3171         -        0

>4406805        /18197

    len =     992   nex =       2

     Term   38065   37812         -        0
     Init   38803   38299         -        0

>4406805        /105341

    len =    1033   nex =       1
```

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```
    Sngl   51271   50239          -        0

>4415905        /38827

  len =     1224   nex =       2

    Init   11227   12103          +        0
    Term   12183   12450          +        0

>4415905        /14564

  len =     1454   nex =       4

    Term    2076    1784          -        0
    Intr    2319    2171          -        0
    Intr    2509    2408          -        0
    Init    2694    2619          -        0

>4415928        /11006

  len =      799   nex =       1

    Sngl   15192   14394          -        0

>4415928        /10850

  len =      819   nex =       2

    Init   34311   34796          +        0
    Term   34892   35129          +        0

>4415928        /25575

  len =     1297   nex =       3

    Term   35365   35116          -        0
    Intr   35883   35801          -        0
    Init   36412   36246          -        0

>4415928        /7802

  len =      550   nex =       1

    Sngl   60672   61212          +        0

>4415928        /33287

  len =      394   nex =       1

    Sngl   60674   61067          +        0

>4417264        /1841

  len =      634   nex =       1

    Sngl   21959   22592          +        0

>4417264        /38497

  len =     1733   nex =       2
```

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```
     Term   50530   49193        -        0
     Init   50925   50620        -        0

>4417264       /31766

    len =     2530   nex =       7

     Term   51442   51135        -        0
     Intr   51661   51539        -        0
     Intr   51937   51791        -        0
     Intr   52307   52197        -        0
     Intr   52643   52499        -        0
     Intr   53125   53032        -        0
     Init   53657   53503        -        0

>4417264       /122848

    len =     1577   nex =       6

     Term   55897   55626        -        0
     Intr   56131   55980        -        0
     Intr   56288   56208        -        0
     Intr   56525   56379        -        0
     Intr   56707   56608        -        0
     Init   57202   57038        -        0

>4417264       /18922

    len =     2470   nex =       9

     Init   82338   82391        +        0
     Intr   82475   82529        +        0
     Intr   82615   82687        +        0
     Intr   82826   82913        +        0
     Intr   83016   83098        +        0
     Intr   83186   83315        +        0
     Intr   83414   83492        +        0
     Intr   83592   83656        +        0
     Term   83736   84101        +        0

>4417264       /33232

    len =     2150   nex =      10

     Init   85855   86098        +        0
     Intr   86197   86292        +        0
     Intr   86405   86479        +        0
     Intr   86591   86659        +        0
     Intr   86902   86978        +        0
     Intr   87066   87154        +        0
     Intr   87232   87361        +        0
     Intr   87439   87529        +        0
     Intr   87622   87687        +        0
     Term   87762   88004        +        0

>4417264       /10672

    len =      892   nex =       2
```

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```
        Term    88380    87971          -        0
        Init    88839    88459          -        0

    >4432811         /9846

      len =     1030    nex =       3

        Init    12818    12984          +        0
        Intr    13233    13498          +        0
        Term    13588    13843          +        0

    >4432829         /119200

      len =      689    nex =       1

        Sngl    14377    13689          -        0

    >4432829         /14334

      len =      511    nex =       1

        Sngl    21464    21974          +        0

    >4432829         /14111

      len =     2126    nex =       2

        Term    37251    36808          -        0
        Init    37474    37342          -        0

    >4432829         /10433

      len =      935    nex =       2

        Term    50590    50253          -        0
        Init    51187    51056          -        0

    >4432829         /3673

      len =      833    nex =       1

        Sngl    69750    70582          +        0

    >4432829         /23166

      len =     3474    nex =       7

        Term    86292    85921          -        0
        Intr    86521    86378          -        0
        Intr    87019    86949          -        0
        Intr    87222    87123          -        0
        Intr    87948    87918          -        0
        Intr    88192    88095          -        0
        Init    89394    89217          -        0

    >4432829         /97776

      len =      790    nex =       1

        Sngl     9122     8339          -        0
```

```
>4432847        /12895

    len =     2510    nex =        0

>4432847        /10730

    len =      676    nex =        2

      Init   55664    55782         +         0
      Term   55970    56339         +         0

>4432847        /97814

    len =      578    nex =        1

      Sngl   60009    60586         +         0

>4454004        /32935

    len =     2261    nex =        7

      Term   17173    16861         -         0
      Intr   17285    17262         -         0
      Intr   17408    17382         -         0
      Intr   17581    17514         -         0
      Intr   17828    17657         -         0
      Intr   18104    17916         -         0
      Init   19121    18868         -         0

>4454004        /29099

    len =     2301    nex =        7

      Term   17173    16834         -         0
      Intr   17285    17262         -         0
      Intr   17408    17382         -         0
      Intr   17581    17514         -         0
      Intr   17828    17657         -         0
      Intr   18104    17916         -         0
      Init   18942    18868         -         0

>4454004        /24398

    len =     2334    nex =        7

      Term   17173    16914         -         0
      Intr   17285    17262         -         0
      Intr   17408    17382         -         0
      Intr   17581    17514         -         0
      Intr   17828    17657         -         0
      Intr   18104    17916         -         0
      Init   18942    18868         -         0

>4454004        /11559

    len =     1123    nex =        4

      Init    2493     2663         +         0
      Intr    2815     2904         +         0
```

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```
    Intr    2993    3115          +        0
    Term    3310    3615          +        0

>4454004       /36536

   len =    1469   nex =       2

    Term   31479   31022          -        0
    Init   32490   31900          -        0

>4454004       /29369

   len =    1586   nex =       3

    Term   34339   33967          -        0
    Intr   34718   34509          -        0
    Init   35552   34914          -        0

>4454004       /38603

   len =    1935   nex =       4

    Init   42043   42575          +        0
    Intr   43023   43266          +        0
    Intr   43356   43461          +        0
    Term   43541   43977          +        0

>4454004       /26380

   len =    1279   nex =       3

    Init     606     927          +        0
    Intr    1278    1392          +        0
    Term    1530    1881          +        0

>4454004       /150586

   len =     714   nex =       1

    Sngl   66988   67701          +        0

>4454004       /60

   len =    1550   nex =       4

    Init   77637   77784          +        0
    Intr   78097   78344          +        0
    Intr   78424   78751          +        0
    Term   78826   79186          +        0

>4454004       /8252

   len =    1648   nex =       1

    Sngl    9384    9995          +        0

>4454022       /23878

   len =    1042   nex =       3
```

```
      Term   11752   11125          -        0
      Intr   11994   11828          -        0
      Init   12166   12098          -        0

>4454022        /40423

     len =    2478   nex =      10

      Init   17803   17967          +        0
      Intr   18546   18615          +        0
      Intr   18803   18927          +        0
      Intr   19076   19178          +        0
      Intr   19253   19349          +        0
      Intr   19432   19497          +        0
      Intr   19591   19729          +        0
      Intr   19823   19902          +        0
      Intr   19987   20091          +        0
      Term   20179   20280          +        0

>4454022        /6527

     len =    1964   nex =       3

      Term   33047   32545          -        0
      Intr   33895   33766          -        0
      Init   34508   34027          -        0

>4454022        /39185

     len =    1852   nex =       5

      Init   35769   36127          +        0
      Intr   36534   36714          +        0
      Intr   36797   36938          +        0
      Intr   37035   37104          +        0
      Term   37198   37620          +        0

>4454022        /14129

     len =    1813   nex =       5

      Init   35826   36127          +        0
      Intr   36534   36714          +        0
      Intr   36797   36938          +        0
      Intr   37035   37104          +        0
      Term   37198   37638          +        0

>4454022        /25397

     len =    1077   nex =       4

      Init   36551   36714          +        0
      Intr   36797   36938          +        0
      Intr   37035   37104          +        0
      Term   37198   37627          +        0

>4454022        /37278

     len =    2619   nex =       9
```

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```
         Term   42843   42630           -         0
         Intr   43042   42943           -         0
         Intr   43257   43130           -         0
         Intr   43493   43326           -         0
         Intr   43710   43595           -         0
         Intr   43953   43801           -         0
         Intr   44188   44045           -         0
         Intr   44554   44268           -         0
         Init   45248   44799           -         0

>4454022        /37786

       len =      310    nex =        1

         Sngl   50741   50436           -         0

>4454022        /108949

       len =     1140    nex =        3

         Term   50767   50437           -         0
         Intr   50907   50807           -         0
         Init   51576   51263           -         0

>4454022        /3802

       len =      233    nex =        1

         Sngl   55269   55037           -         0

>4454022        /41988

       len =     4725    nex =        4

         Term   50767   50548           -         0
         Intr   50907   50807           -         0
         Intr   54477   51263           -         0
         Init   55272   55024           -         0

>4454022        /42101

       len =     1286    nex =        2

         Term   54477   53987           -         0
         Init   55272   55024           -         0

>4454022        /115402

       len =      974    nex =        2

         Term   54477   54301           -         0
         Init   55272   55024           -         0

>4454022        /118240

       len =      939    nex =        2

         Term   54477   54334           -         0
         Init   55272   55024           -         0
```

```
>4454022       /32540

   len =     938    nex =       2

    Term   54477   54335         -        0
    Init   55272   55024         -        0

>4454022       /92204

   len =    4728    nex =       4

    Term   50767   50548         -        0
    Intr   50907   50807         -        0
    Intr   54477   51263         -        0
    Init   55275   55024         -        0

>4454022       /660

   len =     730    nex =       1

    Sngl   57533   56808         -        0

>4454022       /2946

   len =    1489    nex =       3

    Init   67882   68445         +        0
    Intr   68793   68893         +        0
    Term   69004   69370         +        0

>4454022       /21949

   len =    2013    nex =       7

    Init   80581   80861         +        0
    Intr   81338   81412         +        0
    Intr   81487   81543         +        0
    Intr   81634   81800         +        0
    Intr   81901   82035         +        0
    Intr   82128   82330         +        0
    Term   82416   82593         +        0

>4454022       /122649

   len =    1106    nex =       2

    Init   99722   99910         +        0
    Term  100566  100827         +        0

>4454447       /1898

   len =     855    nex =       3

    Init  105950  106053         +        0
    Intr  106164  106220         +        0
    Term  106331  106804         +        0

>4454447       /528

   len =    1665    nex =       2
```

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```
    Term  107093  106729        -        0
    Init  108393  107558        -        0
>4454447       /34908

   len =    2650   nex =       5

    Term  109037  108541        -        0
    Intr  109327  109116        -        0
    Intr  109565  109443        -        0
    Intr  109985  109788        -        0
    Init  111189  110312        -        0

>4454447       /34827

   len =    1645   nex =       3

    Init  118166  118441        +        0
    Intr  118556  118784        +        0
    Term  119096  119810        +        0

>4454447       /39669

   len =    3441   nex =      11

    Term   14000   13685        -        0
    Intr   14573   14085        -        0
    Intr   14892   14646        -        0
    Intr   15056   14980        -        0
    Intr   15442   15368        -        0
    Intr   15656   15555        -        0
    Intr   15876   15758        -        0
    Intr   16151   16028        -        0
    Intr   16297   16247        -        0
    Intr   16489   16391        -        0
    Init   17125   16761        -        0

>4454447       /16840

   len =     730   nex =       1

    Sngl   26481   25758        -        0

>4454447       /15103

   len =     670   nex =       1

    Sngl   28997   29662        +        0

>4454447       /27566

   len =    1108   nex =       5

    Init   29987   30074        +        0
    Intr   30173   30269        +        0
    Intr   30358   30436        +        0
    Intr   30525   30606        +        0
    Term   30777   31079        +        0

>4454447       /15863
```

09555326.061500

```
    len =     3319    nex =       11

     Init    38766    39223         +        0
     Intr    39301    39429         +        0
     Intr    39523    39608         +        0
     Intr    39702    39828         +        0
     Intr    39928    39976         +        0
     Intr    40187    40269         +        0
     Intr    40380    40447         +        0
     Intr    40895    40969         +        0
     Intr    41157    41244         +        0
     Intr    41368    41484         +        0
     Term    41575    42084         +        0

>4454447        /34875

    len =     1750    nex =        5

     Term    42627    42287         -        0
     Intr    42873    42758         -        0
     Intr    43258    43042         -        0
     Intr    43617    43350         -        0
     Init    44030    43729         -        0

>4454447        /2512

    len =      682    nex =        1

     Sngl    48986    49667         +        0

>4454447        /41949

    len =      430    nex =        1

     Sngl    48989    49412         +        0

>4454447        /7215

    len =      566    nex =        1

     Sngl    48989    49554         +        0

>4454447        /120852

    len =      717    nex =        1

     Sngl    48989    49705         +        0

>4454447        /10398

    len =     1539    nex =        2

     Init    61887    62281         +        0
     Term    62817    63409         +        0

>4454447        /91872

    len =     1055    nex =        2
```

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```
       Term   66889   66819          -        0
       Init   67328   67140          -        0

   >4454447        /14736

     len =     970    nex =        2

       Term   66889   66819          -        0
       Init   67371   67140          -        0

   >4454447        /33816

     len =     389    nex =        1

       Sngl   98602   98990          +        0

   >4454585        /10680

     len =    1458    nex =        4

       Init   38285   38437          +        0
       Intr   38805   38874          +        0
       Intr   39093   39133          +        0
       Term   39382   39742          +        0

   >4454587        /105376

     len =     670    nex =        1

       Sngl   68863   69530          +        0

   >4454587        /1268

     len =    2668    nex =       11

       Term   73570   73137          -        0
       Intr   73845   73789          -        0
       Intr   74008   73940          -        0
       Intr   74245   74177          -        0
       Intr   74415   74347          -        0
       Intr   74602   74513          -        0
       Intr   74986   74904          -        0
       Intr   75192   75112          -        0
       Intr   75362   75280          -        0
       Intr   75529   75438          -        0
       Init   75804   75648          -        0

   >4454587        /159035

     len =    1757    nex =        6

       Term   76531   76459          -        0
       Intr   76696   76662          -        0
       Intr   76825   76768          -        0
       Intr   76986   76926          -        0
       Intr   77147   77068          -        0
       Init   77788   77455          -        0

   >4454587        /1045
   len =     2613   nex =        6

    Init   92251   92370         +         0
    Intr   92686   92822         +         0
    Intr   93334   93583         +         0
    Intr   93678   93925         +         0
    Intr   94015   94422         +         0
    Term   94517   94863         +         0

>4455168        /4067

   len =     1477   nex =        2

    Init   39463   39668         +         0
    Term   39795   40939         +         0

>4455168        /279

   len =     1488   nex =        2

    Init   39464   39668         +         0
    Term   40110   40951         +         0

>4455168        /2059

   len =     2265   nex =        8

    Term   41426   41124         -         0
    Intr   41759   41631         -         0
    Intr   41920   41879         -         0
    Intr   42088   42029         -         0
    Intr   42279   42166         -         0
    Intr   42473   42383         -         0
    Intr   42767   42582         -         0
    Init   43388   43192         -         0

>4455168        /17912

   len =     1491   nex =        2

    Init   48010   48378         +         0
    Term   49184   49500         +         0

>4455168        /28717

   len =     1004   nex =        5

    Term   65924   65732         -         0
    Intr   66098   66012         -         0
    Intr   66357   66172         -         0
    Intr   66540   66446         -         0
    Init   66735   66635         -         0

>4455168        /6091

   len =     1847   nex =        5

    Init   71711   71850         +         0
    Intr   72177   72209         +         0
    Intr   72291   72573         +         0
```

```
        Intr    72646    73126          +        0
        Term    73255    73557          +        0

    >4455168        /37329

       len =      326    nex =        1

        Sngl    77591    77916          +        0

    >4455189        /34595

       len =     1423    nex =        2

        Init    16690    17210          +        0
        Term    17612    17935          +        0

    >4455189        /31962

       len =     1362    nex =        3

        Init    16690    16812          +        0
        Intr    17163    17210          +        0
        Term    17612    17944          +        0

    >4455189        /111016

       len =     1319    nex =        3

        Init    16607    16812          +        0
        Intr    17163    17210          +        0
        Term    17612    17925          +        0

    >4455189        /5367

       len =     1551    nex =        5

        Init      184      470          +        0
        Intr      860      924          +        0
        Intr     1005     1077          +        0
        Intr     1179     1232          +        0
        Term     1353     1734          +        0

    >4455189        /35284

       len =     1830    nex =        5

        Term    33174    32856          -        0
        Intr    33398    33270          -        0
        Intr    33645    33478          -        0
        Intr    34173    33730          -        0
        Init    34685    34427          -        0

    >4455189        /2574

       len =     1558    nex =        2

        Init    35777    36240          +        0
        Term    36966    37334          +        0

    >4455189        /33007
```

```
len =     2392    nex =        0

>4455189        /29414

len =     1398    nex =        3

 Init   45338   45550          +        0
 Intr   46160   46309          +        0
 Term   46391   46735          +        0

>4455189        /11679

len =     1606    nex =        6

 Term   51378   51155          -        0
 Intr   51592   51511          -        0
 Intr   51844   51711          -        0
 Intr   52006   51919          -        0
 Intr   52157   52111          -        0
 Init   52760   52482          -        0

>4455189        /93281

len =     1559    nex =        6

 Term   51378   51202          -        0
 Intr   51592   51511          -        0
 Intr   51844   51711          -        0
 Intr   52006   51919          -        0
 Intr   52157   52111          -        0
 Init   52760   52482          -        0

>4455229        /17175

len =     2816    nex =        7

 Init    1117    1501          +        0
 Intr    1589    1775          +        0
 Intr    1866    2116          +        0
 Intr    2667    2781          +        0
 Intr    2897    2989          +        0
 Intr    3429    3572          +        0
 Term    3664    3932          +        0

>4455229        /25830

len =     2596    nex =       10

 Init   16057   16292          +        0
 Intr   16817   16868          +        0
 Intr   16975   17170          +        0
 Intr   17248   17327          +        0
 Intr   17421   17537          +        0
 Intr   17710   17760          +        0
 Intr   17847   17913          +        0
 Intr   18037   18140          +        0
 Intr   18220   18324          +        0
 Term   18417   18652          +        0
```

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```
>4455229       /28535

   len =    1244   nex =       4

    Init   20496   20665        +        0
    Intr   20786   20812        +        0
    Intr   20941   21247        +        0
    Term   21515   21739        +        0

>4455229       /111178

   len =    1125   nex =       1

    Sngl   42529   42245        -        0

>4455229       /31461

   len =    1664   nex =       3

    Term   42587   42245        -        0
    Intr   43426   43256        -        0
    Init   43908   43679        -        0

>4455229       /3059

   len =    1846   nex =       5

    Term   44919   44554        -        0
    Intr   45357   44992        -        0
    Intr   46012   45582        -        0
    Intr   46216   46084        -        0
    Init   46399   46295        -        0

>4455229       /32974

   len =    2037   nex =       6

    Term   47455   46948        -        0
    Intr   47942   47823        -        0
    Intr   48307   48161        -        0
    Intr   48622   48409        -        0
    Intr   48744   48708        -        0
    Init   48984   48865        -        0

>4455229       /16463

   len =    2152   nex =       4

    Init   57208   57329        +        0
    Intr   57866   58020        +        0
    Intr   58096   58362        +        0
    Term   58556   59359        +        0

>4455262       /27914

   len =    2620   nex =      11

    Init  101628  101891        +        0
    Intr  102198  102288        +        0
    Intr  102390  102442        +        0
```

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```
        Intr  102552  102644        +       0
        Intr  102736  102807        +       0
        Intr  103074  103146        +       0
        Intr  103266  103311        +       0
        Intr  103418  103481        +       0
        Intr  103589  103642        +       0
        Intr  103729  103806        +       0
        Term  103880  104247        +       0

>4455262       /4469

      len =    1731   nex =       5

        Init  104601  105127        +       0
        Intr  105304  105424        +       0
        Intr  105524  105750        +       0
        Intr  105860  106150        +       0
        Term  106238  106331        +       0

>4455262       /6952

      len =    2335   nex =       5

        Init  105304  105424        +       0
        Intr  105524  105750        +       0
        Intr  105860  106150        +       0
        Intr  106238  106443        +       0
        Term  106521  106935        +       0

>4455262       /17600

      len =     670   nex =       1

        Sngl   23292   23961        +       0

>4455262       /107817

      len =     299   nex =       1

        Sngl   23641   23939        +       0

>4455262       /11205

      len =     595   nex =       1

        Sngl   76742   77336        +       0

>4455262       /33780

      len =     670   nex =       1

        Sngl   77699   78366        +       0

>4455262       /11383

      len =     110   nex =       1

        Sngl   92430   92539        +       0

>4455262       /18558
```

```
    len =     564   nex =        1

     Sngl   98939   99502         +        0

>4455290        /33360

    len =    1522   nex =        3

     Init   13247   13653         +        0
     Intr   14262   14502         +        0
     Term   14622   14768         +        0

>4455290        /8361

    len =    1930   nex =        7

     Term   34312   34003         -        0
     Intr   34498   34422         -        0
     Intr   34712   34600         -        0
     Intr   35303   35236         -        0
     Intr   35416   35353         -        0
     Intr   35693   35629         -        0
     Init   35927   35782         -        0

>4455290        /15923

    len =    2801   nex =        6

     Init   37303   37639         +        0
     Intr   37723   37815         +        0
     Intr   38133   38237         +        0
     Intr   38889   39041         +        0
     Intr   39597   39695         +        0
     Term   39798   39926         +        0

>4455290        /6626

    len =    1582   nex =        5

     Term    4300    3883         -        0
     Intr    4575    4506         -        0
     Intr    4877    4654         -        0
     Intr    5062    4986         -        0
     Init    5464    5337         -        0

>4455290        /38327

    len =    1909   nex =        6

     Term    4300    3745         -        0
     Intr    4575    4506         -        0
     Intr    4732    4654         -        0
     Intr    4877    4823         -        0
     Intr    5062    4986         -        0
     Init    5653    5337         -        0

>4455290        /8965

    len =     509   nex =        1
```

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```
      Sngl   67245   66737          -        0

>4455290        /40589

     len =     2272   nex =       8

      Term   69364   68891          -        0
      Intr   69544   69452          -        0
      Intr   69755   69618          -        0
      Intr   69969   69842          -        0
      Intr   70213   70053          -        0
      Intr   70386   70296          -        0
      Intr   70653   70597          -        0
      Init   71162   70752          -        0

>4455290        /7191

     len =     1255   nex =       4

      Init   88336   88457          +        0
      Intr   88555   88623          +        0
      Intr   88706   88812          +        0
      Term   88909   89229          +        0

>4455290        /16314

     len =     1256   nex =       4

      Init   88336   88457          +        0
      Intr   88555   88623          +        0
      Intr   88706   88812          +        0
      Term   88909   89230          +        0

>4455290        /124124

     len =      464   nex =       2

      Init   88791   88812          +        0
      Term   88909   89254          +        0

>4455321        /22274

     len =      326   nex =       1

      Sngl   38721   39046          +        0

>4455321        /4369

     len =     2293   nex =       7

      Term   46985   46617          -        0
      Intr   47138   47080          -        0
      Intr   47302   47219          -        0
      Intr   47493   47383          -        0
      Intr   47690   47591          -        0
      Intr   48106   47773          -        0
      Init   48909   48641          -        0

>4455321        /31030
```

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```
      len =     1700    nex =       6

       Term    53798    53289         -         0
       Intr    54066    53875         -         0
       Intr    54274    54158         -         0
       Intr    54493    54413         -         0
       Intr    54739    54643         -         0
       Init    54988    54836         -         0

>4455321        /32761

      len =     2005    nex =       5

       Init    74537    74904         +         0
       Intr    75417    75734         +         0
       Intr    75871    75966         +         0
       Intr    76072    76139         +         0
       Term    76237    76541         +         0

>4455339        /15213

      len =     1956    nex =       2

       Term    56170    55714         -         0
       Init    57669    56842         -         0

>4455339        /36461

      len =     2000    nex =       5

       Term    63860    63223         -         0
       Intr    64081    63950         -         0
       Intr    64602    64187         -         0
       Intr    64869    64736         -         0
       Init    65222    64975         -         0

>4455339        /33995

      len =     1663    nex =       7

       Init    71457    71689         +         0
       Intr    71779    71876         +         0
       Intr    71985    72015         +         0
       Intr    72096    72195         +         0
       Intr    72362    72432         +         0
       Intr    72557    72700         +         0
       Term    72814    73119         +         0

>4455348        /41557

      len =     2280    nex =       4

       Init     1059     1312         +         0
       Intr     2117     2572         +         0
       Intr     2790     2930         +         0
       Term     3044     3338         +         0

>4455348        /32397
```

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```
        len =      919    nex =        1

         Sngl   35039   34121          -         0

>4455348         /31814

        len =     1740    nex =        5

         Term   46233   45987          -         0
         Intr   46420   46319          -         0
         Intr   46639   46514          -         0
         Intr   46883   46799          -         0
         Init   47726   47605          -         0

>4455348         /11590

        len =     1462    nex =        5

         Term   48273   48030          -         0
         Intr   48468   48348          -         0
         Intr   48653   48558          -         0
         Intr   48972   48817          -         0
         Init   49491   49206          -         0

>4455348         /18870

        len =     1078    nex =        2

         Term   53197   52635          -         0
         Init   53712   53462          -         0

>4455348         /39831

        len =     1473    nex =        2

         Term   53197   52607          -         0
         Init   54079   53462          -         0

>4455348         /7873

        len =      550    nex =        1

         Sngl   70379   70404          +         0

>4455348         /42155

        len =     1694    nex =        2

         Term   75967   75455          -         0
         Init   77148   76451          -         0

>4467094         /32072

        len =     1215    nex =        2

         Term  111621  110777          -         0
         Init  111991  111698          -         0

>4467094         /37966
```

```
    len =    1872   nex =       4

     Init  113206  113830        +        0
     Intr  114252  114389        +        0
     Intr  114536  114760        +        0
     Term  114833  115077        +        0

>4467094       /39781

    len =    1570   nex =       3

     Init  115216  115464        +        0
     Intr  116303  116503        +        0
     Term  116590  116779        +        0

>4467094       /38432

    len =    1546   nex =       1

     Sngl  123453  124998        +        0

>4467094       /36909

    len =     715   nex =       2

     Term   18966   18835        -        0
     Init   19549   19051        -        0

>4467094       /33799

    len =    3020   nex =      15

     Term   20057   19801        -        0
     Intr   20251   20141        -        0
     Intr   20444   20333        -        0
     Intr   20602   20525        -        0
     Intr   20750   20685        -        0
     Intr   20936   20832        -        0
     Intr   21221   21024        -        0
     Intr   21392   21300        -        0
     Intr   21572   21480        -        0
     Intr   21781   21657        -        0
     Intr   21937   21861        -        0
     Intr   22163   22042        -        0
     Intr   22345   22259        -        0
     Intr   22567   22517        -        0
     Init   22820   22674        -        0

>4467094       /27692

    len =     678   nex =       2

     Term   57759   57398        -        0
     Init   58075   57842        -        0

>4467094       /42757

    len =    1316   nex =       2

     Term   61776   61514        -        0
```

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```
      Init   62829   62037          -        0

 >4467094       /35382

    len =     654   nex =        1

      Sngl   70975   71628          +        0

 >4467094       /18344

    len =    1002   nex =        4

      Term   71933   71728          -        0
      Intr   72070   72017          -        0
      Intr   72246   72141          -        0
      Init   72729   72335          -        0

 >4467094       /21

    len =    1308   nex =        5

      Term   75122   74915          -        0
      Intr   75276   75205          -        0
      Intr   75455   75351          -        0
      Intr   75807   75617          -        0
      Init   76222   76054          -        0

 >4467094       /7275

    len =     677   nex =        1

      Sngl   89301   88625          -        0

 >4467094       /36035

    len =    1604   nex =        1

      Sngl   90226   88623          -        0

 >4467131       /14711

    len =     954   nex =        5

      Init   16249   16304          +        0
      Intr   16399   16481          +        0
      Intr   16617   16701          +        0
      Intr   16800   16881          +        0
      Term   16973   17202          +        0

 >4467131       /95661

    len =    2264   nex =        0

 >4467131       /34210

    len =    1513   nex =        4

      Term   41365   41312          -        0
      Intr   41555   41457          -        0
      Intr   41673   41645          -        0
```

```
     Init   41919   41805          -        0

>4467131       /27810

    len =    2192   nex =       8

     Term   41365   41180          -        0
     Intr   41555   41457          -        0
     Intr   41673   41645          -        0
     Intr   41919   41805          -        0
     Intr   42078   42013          -        0
     Intr   42823   42739          -        0
     Intr   43052   42917          -        0
     Init   43371   43235          -        0

>4467131       /10238

    len =    1433   nex =       2

     Init   44334   44556          +        0
     Term   45193   45766          +        0

>4467131       /28204

    len =    1390   nex =       2

     Init    4668    5085          +        0
     Term    5231    6052          +        0

>4467131       /6757

    len =    2370   nex =      10

     Term   67293   67054          -        0
     Intr   67447   67379          -        0
     Intr   67646   67546          -        0
     Intr   67826   67730          -        0
     Intr   68190   67911          -        0
     Intr   68614   68402          -        0
     Intr   68808   68694          -        0
     Intr   69041   68936          -        0
     Intr   69249   69136          -        0
     Init   69423   69331          -        0

>4467131       /33640

    len =    2504   nex =       3

     Term   70027   69544          -        0
     Intr   70463   70118          -        0
     Init   72047   70911          -        0

>4467131       /18363

    len =    2290   nex =       7

     Init   73093   73341          +        0
     Intr   73431   73471          +        0
     Intr   73571   73730          +        0
     Intr   73817   73992          +        0
```

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```
        Intr   74069   74173         +        0
        Intr   74253   74399         +        0
        Term   74474   75115         +        0

>4467131        /32152

      len =     1894   nex =       3

        Term   76622   76090         -        0
        Intr   77665   76996         -        0
        Init   77983   77792         -        0

>4467131        /46

      len =     1750   nex =       3

        Term   79829   79698         -        0
        Intr   80958   80259         -        0
        Init   81238   81056         -        0

>4468103        /34690

      len =     1595   nex =       4

        Term   16660   16132         -        0
        Intr   17189   16761         -        0
        Intr   17559   17306         -        0
        Init   17726   17639         -        0

>4468103        /11210

      len =     2187   nex =       2

        Term   42434   41751         -        0
        Init   43937   42613         -        0

>4468103        /36616

      len =     2338   nex =       9

        Term   51987   51673         -        0
        Intr   52425   52370         -        0
        Intr   52655   52555         -        0
        Intr   52938   52846         -        0
        Intr   53108   53030         -        0
        Intr   53260   53199         -        0
        Intr   53449   53356         -        0
        Intr   53625   53537         -        0
        Init   54010   53706         -        0

>4468103        /8606

      len =     2269   nex =       8

        Term   60529   60215         -        0
        Intr   60674   60616         -        0
        Intr   60854   60790         -        0
        Intr   61078   60942         -        0
        Intr   61243   61191         -        0
        Intr   61453   61376         -        0
```

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```
        Intr   61736   61670          -        0
        Init   62483   62229          -        0

    >4468103        /943

      len =     2505   nex =        7

        Init   73001   73391          +        0
        Intr   73776   73852          +        0
        Intr   74018   74152          +        0
        Intr   74290   74344          +        0
        Intr   74437   74524          +        0
        Intr   74795   74893          +        0
        Term   74995   75505          +        0

    >4468801        /41783

      len =      574   nex =        1

        Sngl    1419     846          -        0

    >4468801        /40095

      len =     2263   nex =        2

        Init   35213   36127          +        0
        Term   36724   37475          +        0

    >4468801        /33365

      len =     1750   nex =        2

        Init   48345   49288          +        0
        Term   49374   50086          +        0

    >4468801        /17990

      len =      817   nex =        2

        Term   55584   55278          -        0
        Init   55732   55672          -        0

    >4468801        /121376

      len =      633   nex =        1

        Sngl   80126   79494          -        0

    >4468801        /2583

      len =     1516   nex =        4

        Init   82824   83081          +        0
        Intr   83164   83364          +        0
        Intr   83438   83603          +        0
        Term   83816   84339          +        0

    >4468801        /33704

      len =     1125   nex =        2
    Term   91349   90695        -       0
    Init   91819   91488        -       0

>4468801        /23732

   len =     2181   nex =       7

    Init   94811   95064        +       0
    Intr   95159   95396        +       0
    Intr   95517   95707        +       0
    Intr   95803   96038        +       0
    Intr   96147   96390        +       0
    Intr   96504   96611        +       0
    Term   96769   96991        +       0

>4468976        /92021

   len =     1690   nex =       3

    Init   40307   40379        +       0
    Intr   40472   40537        +       0
    Term   40686   40979        +       0

>4468976        /41011

   len =      640   nex =       1

    Sngl   65865   66504        +       0

>4468976        /207350

   len =      615   nex =       1

    Sngl   88477   89091        +       0

>4469002        /39733

   len =     1604   nex =       1

    Sngl   11302    9699        -       0

>4469002        /7756

   len =      731   nex =       2

    Term   15799   15454        -       0
    Init   16184   15960        -       0

>4469002        /33380

   len =     1150   nex =       2

    Term    1303     530        -       0
    Init    1678    1406        -       0

>4469002        /20829

   len =     3120   nex =       9
```

```
     Term   17041   16572         -        0
     Intr   17196   17137         -        0
     Intr   17550   17459         -        0
     Intr   17871   17773         -        0
     Intr   18146   18054         -        0
     Intr   18282   18225         -        0
     Intr   18836   18687         -        0
     Intr   19200   18957         -        0
     Init   19691   19451         -        0

>4469002       /11048

    len =     701   nex =       2

     Init   20958   21120         +        0
     Term   21217   21658         +        0

>4469002       /799

    len =    2411   nex =       5

     Term   32961   32391         -        0
     Intr   33385   33177         -        0
     Intr   33762   33645         -        0
     Intr   34243   33981         -        0
     Init   34801   34389         -        0

>4469002       /115976

    len =    1533   nex =       4

     Term    3286    2986         -        0
     Intr    3441    3370         -        0
     Intr    3876    3740         -        0
     Init    4518    3982         -        0

>4469002       /8827

    len =     490   nex =       1

     Sngl   61418   61899         +        0

>4469002       /12935

    len =     473   nex =       1

     Sngl   62817   63289         +        0

>4469002       /14002

    len =    1424   nex =       6

     Term   76434   76173         -        0
     Intr   76611   76509         -        0
     Intr   76843   76705         -        0
     Intr   77038   76946         -        0
     Intr   77166   77120         -        0
     Init   77596   77275         -        0

>4469002       /24162
```

```
    len =     1297   nex =       3

     Init   79053   79213          +        0
     Intr   79319   79691          +        0
     Term   79762   80345          +        0

>4469002        /107201

    len =     1003   nex =       2

     Term   84417   83587          -        0
     Init   84589   84496          -        0

>4490291        /30445

    len =      683   nex =       2

     Init   30362   30506          +        0
     Term   30547   30757          +        0

>4490291        /37550

    len =      678   nex =       1

     Sngl   30367   31044          +        0

>4490291        /107617

    len =      730   nex =       3

     Init   35200   35539          +        0
     Intr   35630   35718          +        0
     Term   35791   35920          +        0

>4490291        /119671

    len =     1770   nex =       8

     Term    2571    2225          -        0
     Intr    2837    2755          -        0
     Intr    3098    3032          -        0
     Intr    3230    3180          -        0
     Intr    3408    3296          -        0
     Intr    3574    3461          -        0
     Intr    3724    3668          -        0
     Init    3994    3815          -        0

>4490291        /26806

    len =     1786   nex =       7

     Term    2571    2225          -        0
     Intr    2837    2755          -        0
     Intr    3098    3032          -        0
     Intr    3230    3180          -        0
     Intr    3574    3461          -        0
     Intr    3724    3668          -        0
     Init    3995    3821          -        0
```

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```
>4490291       /32346

   len =    1453   nex =       4

    Init   48142   48411        +       0
    Intr   48498   48686        +       0
    Intr   48784   48946        +       0
    Term   49036   49594        +       0

>4490291       /19165

   len =    1810   nex =       5

    Term   50287   49800        -       0
    Intr   50763   50369        -       0
    Intr   51202   50861        -       0
    Intr   51411   51280        -       0
    Init   51600   51524        -       0

>4490291       /40596

   len =    2940   nex =       9

    Term   69161   68951        -       0
    Intr   69309   69244        -       0
    Intr   69681   69649        -       0
    Intr   69887   69828        -       0
    Intr   70245   70184        -       0
    Intr   70842   70771        -       0
    Intr   71090   71045        -       0
    Intr   71267   71170        -       0
    Init   71890   71358        -       0

>4490291       /24738

   len =    2146   nex =       8

    Term   72209   71984        -       0
    Intr   72366   72294        -       0
    Intr   72656   72456        -       0
    Intr   72877   72736        -       0
    Intr   73193   72966        -       0
    Intr   73516   73293        -       0
    Intr   73720   73603        -       0
    Init   74129   73817        -       0

>4490291       /37432

   len =    2266   nex =       5

    Init   79757   80261        +       0
    Intr   80346   80623        +       0
    Intr   80743   81019        +       0
    Intr   81256   81501        +       0
    Term   81593   82022        +       0

>4490324       /39641

   len =     221   nex =       1
```

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```
    Sngl   18671   18451       -      0

>4490324      /150250

   len =     264   nex =      1

    Sngl   18806   18543       -      0

>4490324      /94851

   len =     264   nex =      1

    Sngl   30283   30027       -      0

>4490324      /41363

   len =     709   nex =      1

    Sngl   30771   30063       -      0

>4490324      /119544

   len =     454   nex =      1

    Sngl   43886   43433       -      0

>4490324      /2315

   len =    1640   nex =      2

    Term   44776   43494       -      0
    Init   45133   44870       -      0

>4490324      /21759

   len =    1815   nex =      6

    Init   57939   58220       +      0
    Intr   58586   58785       +      0
    Intr   58863   59003       +      0
    Intr   59082   59162       +      0
    Intr   59290   59414       +      0
    Term   59498   59753       +      0

>4490324      /37986

   len =    1820   nex =      6

    Init   57939   58220       +      0
    Intr   58586   58785       +      0
    Intr   58863   59003       +      0
    Intr   59082   59162       +      0
    Intr   59290   59414       +      0
    Term   59498   59758       +      0

>4490324      /17181

   len =     812   nex =      1

    Sngl   61230   61139       -      0
```

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```
>4490324       /10140

   len =     550    nex =       1

     Sngl   72094   71548         -        0

>4490324       /14973

   len =     508    nex =       1

     Sngl   72740   73247         +        0

>4490324       /117474

   len =    1555    nex =       5

     Term   82576   82369         -        0
     Intr   82830   82661         -        0
     Intr   83046   82926         -        0
     Intr   83406   83125         -        0
     Init   83798   83526         -        0

>4490701       /6867

   len =    1007    nex =       4

     Term   14504   14298         -        0
     Intr   14711   14599         -        0
     Intr   14909   14780         -        0
     Init   15294   15202         -        0

>4490701       /4374

   len =     986    nex =       4

     Term   14504   14319         -        0
     Intr   14711   14599         -        0
     Intr   14909   14780         -        0
     Init   15294   15202         -        0

>4490701       /41420

   len =    1297    nex =       5

     Term   14504   14300         -        0
     Intr   14711   14599         -        0
     Intr   14909   14780         -        0
     Intr   15303   15202         -        0
     Init   15596   15529         -        0

>4490701       /39825

   len =    2359    nex =       8

     Term   22765   22319         -        0
     Intr   22967   22931         -        0
     Intr   23112   23056         -        0
     Intr   23485   23327         -        0
     Intr   23674   23573         -        0
```

```
      Intr   23973   23864          -         0
      Intr   24351   24252          -         0
      Init   24677   24452          -         0

>4490701        /21752

    len =    2350    nex =       7

      Term   22765   22390          -         0
      Intr   22967   22931          -         0
      Intr   23112   23056          -         0
      Intr   23485   23327          -         0
      Intr   23674   23573          -         0
      Intr   23973   23864          -         0
      Init   24351   24252          -         0

>4490701        /5206

    len =    1848    nex =       5

      Term   25202   24945          -         0
      Intr   25336   25283          -         0
      Intr   25666   25461          -         0
      Intr   25913   25833          -         0
      Init   26792   26587          -         0

>4490717        /8812

    len =    1259    nex =       5

      Term  101847  101489          -         0
      Intr  102100  101932          -         0
      Intr  102370  102201          -         0
      Intr  102632  102454          -         0
      Init  102744  102713          -         0

>4490717        /35272

    len =    3745    nex =      12

      Term  101847  101520          -         0
      Intr  102100  101932          -         0
      Intr  102370  102201          -         0
      Intr  102632  102454          -         0
      Intr  102778  102713          -         0
      Intr  103035  102862          -         0
      Intr  103512  103214          -         0
      Intr  103702  103602          -         0
      Intr  103878  103794          -         0
      Intr  104125  103972          -         0
      Intr  104415  104219          -         0
      Init  105069  104785          -         0

>4490717        /36691

    len =    1469    nex =       3

      Init   66866   67420          +         0
      Intr   67516   67595          +         0
      Term   67696   68334          +         0
```

```
>4490717       /142634

   len =    1930   nex =      4

    Term   74823   74205         -        0
    Intr   75637   75441         -        0
    Intr   75819   75719         -        0
    Init   76126   75910         -        0

>4490717       /8286

   len =    1301   nex =      4

    Term   86445   86257         -        0
    Intr   86683   86641         -        0
    Intr   87034   86928         -        0
    Init   87557   87378         -        0

>4490734       /16697

   len =    1570   nex =      5

    Term   99947   99849         -        0
    Intr  100104  100038         -        0
    Intr  100447  100205         -        0
    Intr  100749  100543         -        0
    Init  101411  100828         -        0

>4490734       /15747

   len =    1796   nex =      5

    Init   37430   37611         +        0
    Intr   37696   37769         +        0
    Intr   38182   38305         +        0
    Intr   38387   38698         +        0
    Term   38786   39225         +        0

>4490734       /20457

   len =     761   nex =      1

    Sngl   42123   42883         +        0

>4490734       /123060

   len =    2073   nex =      7

    Term   46245   45961         -        0
    Intr   46442   46334         -        0
    Intr   46578   46536         -        0
    Intr   46988   46695         -        0
    Intr   47215   47145         -        0
    Intr   47426   47318         -        0
    Init   48033   47855         -        0

>4490734       /37341

   len =    1908   nex =     10
```

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```
          Term   74312   74162        -        0
          Intr   74472   74426        -        0
          Intr   74624   74565        -        0
          Intr   74765   74706        -        0
          Intr   74914   74859        -        0
          Intr   75047   75014        -        0
          Intr   75206   75132        -        0
          Intr   75344   75294        -        0
          Intr   75462   75418        -        0
          Init   76069   75542        -        0

     >4490734        /24741

         len =     2473   nex =        5

          Init   77167   77764        +        0
          Intr   77835   78112        +        0
          Intr   78224   78500        +        0
          Intr   78836   79081        +        0
          Term   79167   79639        +        0

     >4490734        /19562

         len =     1739   nex =        4

          Init   77929   78112        +        0
          Intr   78224   78500        +        0
          Intr   78836   79081        +        0
          Term   79167   79667        +        0

     >4490734        /8545

         len =     1258   nex =        2

          Init   80276   80540        +        0
          Term   80921   81533        +        0

     >4490734        /121755

         len =      936   nex =        1

          Sngl   91697   90762        -        0

     >4490734        /9448

         len =     1168   nex =        3

          Term   93687   93247        -        0
          Intr   93849   93773        -        0
          Init   94003   93932        -        0

     >4490734        /4532

         len =     1270   nex =        3

          Term   93687   93204        -        0
          Intr   93849   93773        -        0
          Init   94003   93932        -        0
```

```
>4490734        /36190

   len =     1275   nex =        4

    Term   93687   93284          -        0
    Intr   93849   93773          -        0
    Intr   94003   93932          -        0
    Init   94558   94467          -        0

>4490734        /2670

   len =     1412   nex =        4

    Term   95295   94885          -        0
    Intr   95484   95394          -        0
    Intr   95663   95587          -        0
    Init   95822   95751          -        0

>4490734        /8313

   len =      741   nex =        1

    Sngl   96873   97152          +        0

>4490734        /22456

   len =      802   nex =        2

    Term   97920   97406          -        0
    Init   98207   98150          -        0

>4510323        /29630

   len =     1699   nex =        8

    Init  116587  116710          +        0
    Intr  116800  116892          +        0
    Intr  117017  117121          +        0
    Intr  117204  117303          +        0
    Intr  117393  117484          +        0
    Intr  117588  117668          +        0
    Intr  117770  117838          +        0
    Term  118019  118285          +        0

>4510323        /30217

   len =     1650   nex =        8

    Init  116589  116710          +        0
    Intr  116800  116892          +        0
    Intr  117017  117121          +        0
    Intr  117204  117303          +        0
    Intr  117393  117484          +        0
    Intr  117588  117668          +        0
    Intr  117770  117838          +        0
    Term  118019  118238          +        0

>4510323        /7542

   len =     1518   nex =        4
```

```
     Term   20387   20042         -         0
     Intr   20566   20495         -         0
     Intr   20825   20709         -         0
     Init   21559   20906         -         0

>4510323       /36518

    len =    2110   nex =       0

>4510323       /16583

    len =     370   nex =       1

     Sngl   53962   53601         -         0

>4510323       /11468

    len =    1887   nex =       7

     Init   56611   56922         +         0
     Intr   57316   57415         +         0
     Intr   57509   57576         +         0
     Intr   57795   57847         +         0
     Intr   57934   57982         +         0
     Intr   58057   58104         +         0
     Term   58233   58497         +         0

>4510323       /28726

    len =    1150   nex =       3

     Term   64164   63855         -         0
     Intr   64685   64611         -         0
     Init   65000   64851         -         0

>4510323       /4776

    len =    1302   nex =       5

     Init   84573   84896         +         0
     Intr   84980   85098         +         0
     Intr   85207   85395         +         0
     Intr   85480   85531         +         0
     Term   85613   85874         +         0

>4510323       /21074

    len =    1418   nex =       3

     Term   85969   85508         -         0
     Intr   86191   86055         -         0
     Init   86925   86535         -         0

>4510323       /13256

    len =    1850   nex =       3

     Term   94960   94737         -         0
     Intr   95223   95079         -         0
```

09595326.061600

```
    Init   96586   95450         -       0

>4510338       /96414

   len =    1937   nex =       3

    Term   28550   28235         -       0
    Intr   29151   29012         -       0
    Init   30171   29548         -       0

>4510338       /24255

   len =     971   nex =       1

    Sngl   34528   33558         -       0

>4510338       /2576

   len =    2237   nex =       9

    Init   36933   37151         +       0
    Intr   37427   37542         +       0
    Intr   37638   37697         +       0
    Intr   37834   38016         +       0
    Intr   38097   38201         +       0
    Intr   38291   38387         +       0
    Intr   38479   38586         +       0
    Intr   38668   38803         +       0
    Term   38888   39169         +       0

>4510338       /156731

   len =    1390   nex =       2

    Init   44273   44363         +       0
    Term   45034   45662         +       0

>4510338       /114613

   len =    1210   nex =       0

>4510338       /5167

   len =    2170   nex =       5

    Init   52854   52965         +       0
    Intr   53527   53789         +       0
    Intr   53878   54089         +       0
    Intr   54172   54439         +       0
    Term   54516   55019         +       0

>4510338       /3668

   len =    1690   nex =       2

    Init   56164   56327         +       0
    Term   57130   57851         +       0

>4510338       /2813
```

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```
     len =    1832   nex =       7

      Init   68165   68340        +        0
      Intr   68439   68646        +        0
      Intr   68750   68835        +        0
      Intr   68919   69020        +        0
      Intr   69117   69250        +        0
      Intr   69373   69421        +        0
      Term   69637   69988        +        0

>4510338       /12251

     len =    1059   nex =       1

      Sngl   71208   70150        -        0

>4510360       /39036

     len =    2077   nex =       8

      Term  101338  101020        -        0
      Intr  101452  101406        -        0
      Intr  101719  101533        -        0
      Intr  102088  101798        -        0
      Intr  102369  102175        -        0
      Intr  102562  102461        -        0
      Intr  102944  102645        -        0
      Init  103096  103017        -        0

>4510360       /36041

     len =    1536   nex =       5

      Term  102088  101812        -        0
      Intr  102369  102175        -        0
      Intr  102562  102461        -        0
      Intr  102944  102645        -        0
      Init  103095  103017        -        0

>4510360       /21707

     len =    2395   nex =       7

      Term  101338  101025        -        0
      Intr  101452  101406        -        0
      Intr  101719  101533        -        0
      Intr  102088  101798        -        0
      Intr  102369  102175        -        0
      Intr  102562  102461        -        0
      Init  102944  102645        -        0

>4510360       /28577

     len =    1009   nex =       3

      Term  103942  103748        -        0
      Intr  104361  104019        -        0
      Init  104756  104575        -        0

>4510360       /22535
```

```
     len =    1330   nex =       3

      Term  105113  104790         -        0
      Intr  105546  105210         -        0
      Init  106116  105944         -        0

>4510360       /39503

     len =    1090   nex =       3

      Term  107026  106783         -        0
      Intr  107457  107118         -        0
      Init  107872  107683         -        0

>4510360       /29951

     len =    1349   nex =       4

      Init   12868   13060         +        0
      Intr   13411   13550         +        0
      Intr   13677   13802         +        0
      Term   13876   14216         +        0

>4510360       /13310

     len =    2350   nex =       8

      Init   31440   31565         +        0
      Intr   31864   31919         +        0
      Intr   32001   32107         +        0
      Intr   32534   32590         +        0
      Intr   32698   32761         +        0
      Intr   32865   32999         +        0
      Intr   33109   33192         +        0
      Term   33299   33781         +        0

>4510360       /26418

     len =     874   nex =       1

      Sngl   46437   45564         -        0

>4510360       /19481

     len =     806   nex =       1

      Sngl   49946   49141         -        0

>4510360       /91908

     len =     732   nex =       1

      Sngl    5525    6256         +        0

>4510392       /16423

     len =    1588   nex =       2

      Term   35088   34032         -        0
```

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```
     Intr   46949   47090         +        0
     Intr   47177   47246         +        0
     Intr   47334   47399         +        0
     Intr   47531   47653         +        0
     Term   47737   48053         +        0

>4512646       /41045

    len =    1632   nex =       2

     Term   48617   48420         -        0
     Init   50051   49809         -        0

>4512646       /40190

    len =    1430   nex =       2

     Term   49533   49412         -        0
     Init   50053   49809         -        0

>4512646       /96543

    len =    1630   nex =       3

     Term   48617   48428         -        0
     Intr   49533   49412         -        0
     Init   50057   49809         -        0

>4512646       /15689

    len =    1669   nex =       3

     Term   48617   48425         -        0
     Intr   49533   49412         -        0
     Init   50093   49809         -        0

>4512646       /41317

    len =    2050   nex =       8

     Term   50564   50329         -        0
     Intr   50835   50657         -        0
     Intr   51046   50938         -        0
     Intr   51222   51137         -        0
     Intr   51577   51518         -        0
     Intr   51893   51678         -        0
     Intr   52086   51973         -        0
     Init   52376   52179         -        0

>4512656       /41712

    len =    1391   nex =       4

     Term  105749  105453         -        0
     Intr  105920  105838         -        0
     Intr  106277  106194         -        0
     Init  106843  106633         -        0

>4512656       /125409
```

```
    len =      971    nex =        1

     Sngl   14193    15163         +         0

>4512656         /35051

    len =     2301    nex =        7

     Term   23482    23178         -         0
     Intr   23695    23596         -         0
     Intr   24031    23903         -         0
     Intr   24198    24130         -         0
     Intr   24399    24304         -         0
     Intr   24814    24499         -         0
     Init   25478    25178         -         0

>4512656         /102453

    len =      581    nex =        1

     Sngl   44496    45076         +         0

>4512656         /42863

    len =     1078    nex =        2

     Term   47194    46904         -         0
     Init   47981    47309         -         0

>4512656         /41214

    len =     1304    nex =        3

     Init   54263    54446         +         0
     Intr   54531    54981         +         0
     Term   55077    55566         +         0

>4512656         /41345

    len =     1301    nex =        2

     Init   54266    54981         +         0
     Term   55077    55566         +         0

>4512656         /99303

    len =     1363    nex =        3

     Init   54266    54446         +         0
     Intr   54876    54981         +         0
     Term   55077    55628         +         0

>4512656         /156913

    len =      550    nex =        1

     Sngl   54268    54446         +         0

>4512656         /157512
```

```
    len =     265    nex =       1

     Sngl   55360   55624         +        0

>4512656        /35604

    len =    2847    nex =       3

     Term   58659   58568         -        0
     Intr   58861   58780         -        0
     Init   61159   60918         -        0

>4512656        /17416

    len =    3176    nex =      13

     Init   75697   75985         +        0
     Intr   76075   76255         +        0
     Intr   76358   76443         +        0
     Intr   76651   76755         +        0
     Intr   76872   76934         +        0
     Intr   77055   77164         +        0
     Intr   77316   77435         +        0
     Intr   77507   77582         +        0
     Intr   77677   77750         +        0
     Intr   77840   77960         +        0
     Intr   78076   78173         +        0
     Intr   78255   78378         +        0
     Term   78474   78872         +        0

>4512656        /21855

    len =    1706    nex =       5

     Init   81740   82100         +        0
     Intr   82425   82580         +        0
     Intr   82665   82797         +        0
     Intr   82880   82919         +        0
     Term   83024   83445         +        0

>4512656        /2928

    len =    1721    nex =       5

     Init   81740   82100         +        0
     Intr   82425   82580         +        0
     Intr   82665   82797         +        0
     Intr   82880   82919         +        0
     Term   83024   83460         +        0

>4512656        /26194

    len =     610    nex =       1

     Sngl   84529   85135         +        0

>4512656        /17723

    len =    1334    nex =       5
```

```
      Term   88998   88678        -        0
      Intr   89288   89093        -        0
      Intr   89677   89635        -        0
      Intr   89871   89764        -        0
      Init   90011   89950        -        0

 >4512656        /2227

    len =     1692   nex =       3

      Term   98186   98145        -        0
      Intr   98404   98327        -        0
      Init   99420   98801        -        0

 >4512690        /91902

    len =     1930   nex =       4

      Init   13553   13670        +        0
      Intr   13990   14118        +        0
      Intr   14808   14995        +        0
      Term   15104   15473        +        0

 >4512690        /20429

    len =     3383   nex =      11

      Term   22804   22366        -        0
      Intr   23013   22893        -        0
      Intr   23189   23121        -        0
      Intr   23389   23266        -        0
      Intr   23599   23484        -        0
      Intr   24010   23879        -        0
      Intr   24292   24222        -        0
      Intr   24490   24406        -        0
      Intr   24696   24611        -        0
      Intr   24878   24814        -        0
      Init   25461   25365        -        0

 >4512690        /36904

    len =     2530   nex =      11

      Term    4054    3778        -        0
      Intr    4266    4144        -        0
      Intr    4517    4353        -        0
      Intr    4741    4607        -        0
      Intr    4977    4825        -        0
      Intr    5158    5084        -        0
      Intr    5345    5268        -        0
      Intr    5505    5441        -        0
      Intr    5714    5603        -        0
      Intr    5907    5797        -        0
      Init    6307    6091        -        0

 >4512690        /30611

    len =     1786   nex =       5

      Init   72103   72432        +        0
```

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```
      Intr   72543   72616         +         0
      Intr   73261   73335         +         0
      Intr   73495   73521         +         0
      Term   73686   73888         +         0

>4512690        /27629

    len =      970   nex =        3

      Term   74223   74064         -         0
      Intr   74366   74301         -         0
      Init   75026   74717         -         0

>4512690        /6967

    len =     2086   nex =        5

      Term   84416   84137         -         0
      Intr   84712   84524         -         0
      Intr   84919   84815         -         0
      Intr   85308   85275         -         0
      Init   86222   85706         -         0

>4512690        /13277

    len =     2026   nex =        7

      Term    7610    7394         -         0
      Intr    8243    7704         -         0
      Intr    8397    8338         -         0
      Intr    8529    8479         -         0
      Intr    8713    8646         -         0
      Intr    8966    8823         -         0
      Init    9212    9088         -         0

>4519183        /205761

    len =     1035   nex =        2

      Init   26007   26147         +         0
      Term   26837   27041         +         0

>4519183        /3325

    len =     1098   nex =        3

      Init   31470   31599         +         0
      Intr   31784   31867         +         0
      Term   32242   32567         +         0

>4519183        /25308

    len =     1078   nex =        3

      Init   31473   31599         +         0
      Intr   31784   31867         +         0
      Term   32242   32550         +         0

>4519183        /111672
```

```
    len =     1314    nex =        7

      Init    42016    42331        +        0
      Intr    42423    42478        +        0
      Intr    42633    42692        +        0
      Intr    42768    42845        +        0
      Intr    42937    43066        +        0
      Intr    43141    43193        +        0
      Term    43279    43329        +        0

>4519183        /3201

    len =     1104    nex =        0

>4519183        /36752

    len =      977    nex =        3

      Init    55410    55539        +        0
      Intr    55654    55936        +        0
      Term    56039    56386        +        0

>4519183        /21404

    len =     2255    nex =        6

      Init    65304    65895        +        0
      Intr    65972    66016        +        0
      Intr    66102    66215        +        0
      Intr    66686    66766        +        0
      Intr    67046    67213        +        0
      Term    67358    67558        +        0

>4519183        /2875

    len =      251    nex =        1

      Sngl    73445    73195        -        0

>4519186        /34522

    len =      574    nex =        1

      Sngl    11854    12427        +        0

>4519187        /20618

    len =     1873    nex =        5

      Init    26310    26574        +        0
      Intr    26838    27409        +        0
      Intr    27503    27565        +        0
      Intr    27662    27713        +        0
      Term    27800    28182        +        0

>4519187        /14357

    len =     1270    nex =        3

      Term    31639    31240        -        0
```

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```
     Intr   32336   32265         -        0
     Init   32505   32367         -        0

>4519187       /104871

    len =     350   nex =       1

     Sngl   32958   32609         -        0

>4519187       /2924

    len =    1734   nex =       3

     Term   31639   31291         -        0
     Intr   32336   32265         -        0
     Init   33024   32367         -        0

>4519187       /29696

    len =    1707   nex =       7

     Init   48852   48942         +        0
     Intr   49200   49468         +        0
     Intr   49539   49643         +        0
     Intr   49730   49816         +        0
     Intr   49902   49960         +        0
     Intr   50084   50237         +        0
     Term   50302   50558         +        0

>4519187       /25264

    len =    2718   nex =       8

     Init   53157   53318         +        0
     Intr   53816   53957         +        0
     Intr   54090   54186         +        0
     Intr   54263   54332         +        0
     Intr   54428   54532         +        0
     Intr   54613   54711         +        0
     Intr   55041   55128         +        0
     Term   55241   55522         +        0

>4519188       /17098

    len =     850   nex =       2

     Term   14912   14578         -        0
     Init   15424   14997         -        0

>4519188       /95892

    len =    1436   nex =       6

     Init   29371   29651         +        0
     Intr   29739   29792         +        0
     Intr   29890   29970         +        0
     Intr   30055   30124         +        0
     Intr   30331   30419         +        0
     Term   30560   30806         +        0
```

```
>4519188       /251

   len =    1462   nex =       6

    Init   29379   29651        +        0
    Intr   29739   29792        +        0
    Intr   29890   29970        +        0
    Intr   30055   30124        +        0
    Intr   30331   30419        +        0
    Term   30560   30840        +        0

>4519190       /158397

   len =    1101   nex =       2

    Init   31318   31483        +        0
    Term   31775   32418        +        0

>4519190       /15702

   len =    3270   nex =       7

    Init   37427   38279        +        0
    Intr   38619   38903        +        0
    Intr   38977   39195        +        0
    Intr   39285   39464        +        0
    Intr   39540   40031        +        0
    Intr   40123   40284        +        0
    Term   40365   40696        +        0

>4519191       /26549

   len =     734   nex =       2

    Init   35516   35748        +        0
    Term   35845   36249        +        0

>4519191       /35233

   len =    1554   nex =       2

    Term   37318   36349        -        0
    Init   37902   37399        -        0

>4519191       /5748

   len =     730   nex =       2

    Term   38416   37997        -        0
    Init   38725   38497        -        0

>4519191       /97675

   len =    1914   nex =       4

    Init   45399   45522        +        0
    Intr   45674   45748        +        0
    Intr   46594   46904        +        0
    Term   47000   47312        +        0
```

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```
>4519191        /17006

   len =    2118   nex =       3

    Init   47579   48385        +        0
    Intr   48874   49077        +        0
    Term   49162   49696        +        0

>4519191        /6244

   len =    3780   nex =      13

    Init   59272   59472        +        0
    Intr   59565   59603        +        0
    Intr   59866   60033        +        0
    Intr   60122   60227        +        0
    Intr   60403   60569        +        0
    Intr   60679   60921        +        0
    Intr   61199   61287        +        0
    Intr   61732   61782        +        0
    Intr   61874   61964        +        0
    Intr   62161   62265        +        0
    Intr   62359   62429        +        0
    Intr   62521   62616        +        0
    Term   62794   63051        +        0

>4519192        /2467

   len =     407   nex =       1

    Sngl   23640   24046        +        0

>4519192        /37019

   len =    2212   nex =       8

    Init   34427   34741        +        0
    Intr   34950   35008        +        0
    Intr   35106   35180        +        0
    Intr   35255   35368        +        0
    Intr   35462   35532        +        0
    Intr   35652   35949        +        0
    Intr   36042   36331        +        0
    Term   36419   36638        +        0

>4519192        /5819

   len =     771   nex =       1

    Sngl    3537    2767        -        0

>4519192        /29301

   len =    2235   nex =      11

    Init   39652   39840        +        0
    Intr   40077   40138        +        0
    Intr   40278   40380        +        0
    Intr   40499   40557        +        0
    Intr   40641   40664        +        0
    Intr   40784   40866          +        0
    Intr   40968   41032          +        0
    Intr   41147   41196          +        0
    Intr   41287   41388          +        0
    Intr   41473   41532          +        0
    Term   41634   41886          +        0

>4519192        /21955

  len =     2209   nex =        6

    Term   46248   45906          -        0
    Intr   46484   46326          -        0
    Intr   47033   46716          -        0
    Intr   47390   47130          -        0
    Intr   47555   47478          -        0
    Init   48114   47633          -        0

>4519192        /533

  len =     1489   nex =        5

    Term   48529   48236          -        0
    Intr   48841   48619          -        0
    Intr   49138   48966          -        0
    Intr   49404   49245          -        0
    Init   49705   49484          -        0

>4519192        /31447

  len =     1930   nex =        7

    Init   76620   76794          +        0
    Intr   77019   77233          +        0
    Intr   77315   77472          +        0
    Intr   77567   77754          +        0
    Intr   77855   78107          +        0
    Intr   78180   78328          +        0
    Term   78423   78546          +        0

>4519192        /33530

  len =     1896   nex =        6

    Init     861    1342          +        0
    Intr    1549    1603          +        0
    Intr    1757    1818          +        0
    Intr    1914    2020          +        0
    Intr    2119    2206          +        0
    Term    2324    2756          +        0

>4519193        /22588

  len =     1210   nex =        1

    Sngl   10581   11344          +        0

>4519193        /12734

  len =     3093   nex =       15
```

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```
     Term   11783    11499          -         0
     Intr   11954    11871          -         0
     Intr   12113    12043          -         0
     Intr   12282    12213          -         0
     Intr   12459    12367          -         0
     Intr   12649    12556          -         0
     Intr   12798    12737          -         0
     Intr   12989    12945          -         0
     Intr   13146    13084          -         0
     Intr   13398    13333          -         0
     Intr   13650    13534          -         0
     Intr   13862    13742          -         0
     Intr   14095    13965          -         0
     Intr   14272    14240          -         0
     Init   14591    14446          -         0

>4519193        /2934

    len =      610    nex =        1

     Sngl   29932    29326          -         0

>4519193        /101342

    len =      310    nex =        1

     Sngl   43149    43454          +         0

>4519193        /31309

    len =      763    nex =        1

     Sngl   51604    50842          -         0

>4519193        /38326

    len =     2324    nex =        6

     Term   75991    75658          -         0
     Intr   76453    76077          -         0
     Intr   76876    76829          -         0
     Intr   77135    76973          -         0
     Intr   77307    77229          -         0
     Init   77981    77783          -         0

>4519193        /114703

    len =      576    nex =        1

     Sngl    7839     8414          +         0

>4519194        /20539

    len =     1577    nex =        3

     Init   15272    15482          +         0
     Intr   15570    15615          +         0
     Term   16443    16848          +         0
```

```
>4519194        /91757

   len =    1407   nex =        7

    Init   16952   17134        +        0
    Intr   17217   17260        +        0
    Intr   17389   17484        +        0
    Intr   17694   17786        +        0
    Intr   17919   18006        +        0
    Intr   18095   18156        +        0
    Term   18245   18358        +        0

>4519194        /32257

   len =     910   nex =        1

    Sngl   41773   40872        -        0

>4519194        /9170

   len =    1599   nex =        4

    Term   48741   48223        -        0
    Intr   48949   48918        -        0
    Intr   49210   49075        -        0
    Init   49821   49647        -        0

>4519194        /36525

   len =    1953   nex =        1

    Sngl   59919   61346        +        0

>4519194        /7233

   len =    1270   nex =        4

    Term   68594   68376        -        0
    Intr   68973   68686        -        0
    Intr   69272   69107        -        0
    Init   69644   69537        -        0

>4519194        /819

   len =    1270   nex =        4

    Init    7404    7643        +        0
    Intr    7741    8080        +        0
    Intr    8177    8230        +        0
    Term    8376    8673        +        0

>4519194        /6181

   len =    1810   nex =        6

    Init   74654   74757        +        0
    Intr   75098   75146        +        0
    Intr   75472   75579        +        0
    Intr   75770   75884        +        0
    Intr   75966   76083        +        0
```

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```
      Term    76205   76459          +        0

 >4519194        /13954

     len =     1019    nex =        2

      Init     9367    9653          +        0
      Term     9736   10385          +        0

 >4519195        /26815

     len =     3889    nex =       16

      Term    12207   11831          -        0
      Intr    12457   12314          -        0
      Intr    12656   12530          -        0
      Intr    12841   12762          -        0
      Intr    13213   12962          -        0
      Intr    13440   13312          -        0
      Intr    13706   13657          -        0
      Intr    13944   13915          -        0
      Intr    14169   14056          -        0
      Intr    14435   14374          -        0
      Intr    14556   14514          -        0
      Intr    14691   14636          -        0
      Intr    14893   14836          -        0
      Intr    15044   14976          -        0
      Intr    15431   15352          -        0
      Init    15719   15536          -        0

 >4519195        /17362

     len =     2377    nex =        6

      Term    24714   24441          -        0
      Intr    24903   24813          -        0
      Intr    25545   25430          -        0
      Intr    26012   25683          -        0
      Intr    26589   26090          -        0
      Init    26817   26772          -        0

 >4519195        /40420

     len =     1753    nex =        6

      Init    42376   42569          +        0
      Intr    42664   42895          +        0
      Intr    42979   43142          +        0
      Intr    43253   43535          +        0
      Intr    43622   43788          +        0
      Term    43875   44128          +        0

 >4519195        /94524

     len =      475    nex =        2

      Init    43654   43788          +        0
      Term    43875   44128          +        0

 >4519195        /20767
```

```
    len =    1874   nex =       6

     Term   47286   46916        -        0
     Intr   47435   47386        -        0
     Intr   47604   47531        -        0
     Intr   48033   47989        -        0
     Intr   48249   48134        -        0
     Init   48789   48477        -        0

>4519195       /97350

    len =     613   nex =       2

     Term   67803   67627        -        0
     Init   68239   67907        -        0

>4519197       /32643

    len =    1763   nex =       1

     Sngl   27667   25905        -        0

>4521999       /98976

    len =     430   nex =       1

     Sngl   10245   10673        +        0

>4521999       /109109

    len =     399   nex =       1

     Sngl   10295   10686        +        0

>4521999       /106320

    len =     278   nex =       1

     Sngl   10379   10656        +        0

>4521999       /115957

    len =     295   nex =       1

     Sngl   10445   10739        +        0

>4521999       /114949

    len =     285   nex =       1

     Sngl   10447   10731        +        0

>4521999       /10836

    len =     562   nex =       1

     Sngl   12451   11890        -        0

>4521999       /151572
```

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```
   len =    1914   nex =       6

    Term   22290   22087         -        0
    Intr   22510   22406         -        0
    Intr   22867   22604         -        0
    Intr   23125   22964         -        0
    Intr   23303   23226         -        0
    Init   24000   23670         -        0

>4521999       /31655

   len =    1630   nex =       5

    Term   26057   25794         -        0
    Intr   26219   26143         -        0
    Intr   26450   26332         -        0
    Intr   26899   26820         -        0
    Init   27415   27008         -        0

>4521999       /92908

   len =    2017   nex =       4

    Term   31465   31171         -        0
    Intr   31816   31636         -        0
    Intr   32453   32089         -        0
    Init   33187   33057         -        0

>4521999       /34062

   len =    1139   nex =       3

    Term   38591   38301         -        0
    Intr   38843   38709         -        0
    Init   39439   39192         -        0

>4521999       /3948

   len =    1096   nex =       1

    Sngl    9611    9785         +        0

>4521999       /95453

   len =    1044   nex =       3

    Init    9613    9785         +        0
    Intr   10095   10272         +        0
    Term   10295   10656         +        0

>4521999       /17347

   len =    1074   nex =       1

    Sngl    9613   10686         +        0

>4521999       /125485

   len =    1115   nex =       2
```

```
        Init    9613    9785          +        0
        Term   10095   10272          +        0

>4521999        /125977

      len =     1120   nex =        3

        Init    9613    9785          +        0
        Intr   10095   10272          +        0
        Term   10295   10732          +        0

>4521999        /98855

      len =     1126   nex =        3

        Init    9613    9785          +        0
        Intr   10095   10272          +        0
        Term   10295   10738          +        0

>4521999        /7420

      len =      986   nex =        2

        Init    9615    9785          +        0
        Term   10095   10600          +        0

>4521999        /234

      len =     1118   nex =        2

        Init    9615    9785          +        0
        Term   10095   10272          +        0

>4521999        /26796

      len =     1119   nex =        3

        Init    9615    9785          +        0
        Intr   10095   10272          +        0
        Term   10295   10733          +        0

>4521999        /13879

      len =     1121   nex =        2

        Init    9615    9785          +        0
        Term   10095   10735          +        0

>4522002        /18876

      len =     2097   nex =        4

        Init   31368   31702          +        0
        Intr   31777   31877          +        0
        Intr   32003   32211          +        0
        Term   32776   33464          +        0

>4522002        /8550
```

```
    len =     550   nex =       1

     Sngl   36522   35979         -        0

>4522002        /10080

    len =    2566   nex =       7

     Term   37918   37569         -        0
     Intr   38093   38029         -        0
     Intr   38349   38274         -        0
     Intr   38569   38442         -        0
     Intr   38766   38659         -        0
     Intr   39324   39168         -        0
     Init   40134   39924         -        0

>4522002        /35371

    len =    1570   nex =       3

     Term   70494   70283         -        0
     Intr   70779   70573         -        0
     Init   71849   71563         -        0

>4531433        /142861

    len =     940   nex =       1

     Sngl   33855   32916         -        0

>4531433        /16421

    len =    1991   nex =       8

     Term   35017   34361         -        0
     Intr   35195   35098         -        0
     Intr   35388   35286         -        0
     Intr   35569   35484         -        0
     Intr   35771   35684         -        0
     Intr   35976   35863         -        0
     Intr   36161   36073         -        0
     Init   36341   36247         -        0

>4531433        /41669

    len =     276   nex =       1

     Sngl   37215   36940         -        0

>4538895        /7395

    len =    2470   nex =       6

     Term   47375   47093         -        0
     Intr   47611   47463         -        0
     Intr   47817   47700         -        0
     Intr   48199   48007         -        0
     Intr   48407   48286         -        0
     Init   49561   49113         -        0
```

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```
>4538895        /8795

    len =     756    nex =       1

      Sngl   49466   48947         -        0

>4538895        /24071

    len =    1392    nex =       3

      Init   72494   72654         +        0
      Intr   72892   73080         +        0
      Term   73174   73422         +        0

>4538895        /27800

    len =     790    nex =       3

      Init   72493   72654         +        0
      Intr   72892   73080         +        0
      Term   73174   73280         +        0

>4538895        /19582

    len =    1899    nex =       2

      Term    6321    5522         -        0
      Init    7420    6901         -        0

>4538895        /25257

    len =    1939    nex =       2

      Term    6321    5516         -        0
      Init    7454    6901         -        0

>4538895        /142381

    len =    1954    nex =       3

      Init   87087   87340         +        0
      Intr   88405   88485         +        0
      Term   88572   89040         +        0

>4538918        /33495

    len =    2564    nex =       6

      Init   35043   35417         +        0
      Intr   35652   35904         +        0
      Intr   36064   36212         +        0
      Intr   36295   36552         +        0
      Intr   36711   36902         +        0
      Term   37042   37606         +        0

>4538918        /15410

    len =    1705    nex =       4

      Term   52803   52534         -        0
```

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```
    Intr   53006   52889         -         0
    Intr   53214   53100         -         0
    Init   53440   53336         -         0

>4538918        /31259

   len =    2096   nex =       7

    Init   55471   55645         +         0
    Intr   56096   56230         +         0
    Intr   56317   56541         +         0
    Intr   56641   56823         +         0
    Intr   56932   57155         +         0
    Intr   57239   57284         +         0
    Term   57378   57566         +         0

>4538918        /34934

   len =    3130   nex =      12

    Init   70373   70535         +         0
    Intr   70658   70701         +         0
    Intr   70897   70992         +         0
    Intr   71153   71245         +         0
    Intr   71398   71488         +         0
    Intr   71582   71643         +         0
    Intr   71733   71917         +         0
    Intr   72056   72233         +         0
    Intr   72312   72355         +         0
    Intr   72493   72682         +         0
    Intr   72788   72805         +         0
    Term   73209   73502         +         0

>4538918        /7421

   len =    1241   nex =       1

    Sngl   79061   77821         -         0

>4538918        /148597

   len =    1482   nex =       4

    Init   93052   93292         +         0
    Intr   93665   93847         +         0
    Intr   94012   94176         +         0
    Term   94312   94533         +         0

>4538949        /13231

   len =     738   nex =       3

    Init   52100   52260         +         0
    Intr   52368   52518         +         0
    Term   52605   52837         +         0

>4538949        /39765

   len =    1486   nex =       6
```

09555326.061602

```
        Init   53668   53951         +        0
        Intr   54035   54119         +        0
        Intr   54209   54259         +        0
        Intr   54358   54478         +        0
        Intr   54585   54719         +        0
        Term   54798   55153         +        0

    >4538949      /93802

       len =    310   nex =       1

        Sngl   54895   55195         +        0

    >4538949      /1354

       len =   1825   nex =       7

        Init   66408   66496         +        0
        Intr   66590   66728         +        0
        Intr   66808   66882         +        0
        Intr   67001   67122         +        0
        Intr   67205   67280         +        0
        Intr   67362   67421         +        0
        Term   67521   67843         +        0

    >4538949      /36655

       len =   3584   nex =       4

        Term   77287   76801         -        0
        Intr   77426   77365         -        0
        Intr   78110   77691         -        0
        Init   80384   79836         -        0

    >4538972      /6822

       len =   2801   nex =      13

        Term   11535   11345         -        0
        Intr   11698   11621         -        0
        Intr   11841   11769         -        0
        Intr   12019   11939         -        0
        Intr   12214   12164         -        0
        Intr   12391   12297         -        0
        Intr   12711   12631         -        0
        Intr   12872   12788         -        0
        Intr   13313   13234         -        0
        Intr   13530   13416         -        0
        Intr   13703   13633         -        0
        Intr   13870   13786         -        0
        Init   14145   13945         -        0

    >4538972      /41992

       len =   2052   nex =       5

        Init   32316   32561         +        0
        Intr   32643   32715         +        0
        Intr   33489   33697         +        0
        Intr   33789   33935         +        0
```

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```
    Term   34034   34367         +        0

>4538972        /35962

   len =     823   nex =        1

    Sngl   35958   36389         +        0

>4538972        /31115

   len =    1478   nex =        3

    Init    3879    4068         +        0
    Intr    4258    4391         +        0
    Term    5063    5356         +        0

>4538990        /32791

   len =    4390   nex =        8

    Term   22701   22300         -        0
    Intr   22820   22779         -        0
    Intr   22937   22896         -        0
    Intr   23128   23029         -        0
    Intr   23284   23223         -        0
    Intr   23456   23375         -        0
    Intr   25216   25034         -        0
    Init   26686   26545         -        0

>4538990        /36491

   len =     533   nex =        1

    Sngl    4200    4732         +        0

>4538990        /40182

   len =    2574   nex =        8

    Term   47130   47007         -        0
    Intr   47313   47244         -        0
    Intr   47449   47400         -        0
    Intr   47627   47560         -        0
    Intr   47778   47717         -        0
    Intr   47991   47868         -        0
    Intr   48467   48429         -        0
    Init   48934   48614         -        0

>4538990        /6718

   len =    2984   nex =       12

    Term   56364   56049         -        0
    Intr   56633   56462         -        0
    Intr   56834   56722         -        0
    Intr   57028   56963         -        0
    Intr   57314   57213         -        0
    Intr   57443   57391         -        0
    Intr   57592   57535         -        0
    Intr   57780   57682         -        0
```

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```
    Intr   57898   57855        -         0
    Intr   58299   58209        -         0
    Intr   58481   58455        -         0
    Init   59032   58791        -         0

>4539290       /9546

   len =    1353   nex =       3

    Init   31019   31261        +         0
    Intr   31721   31841        +         0
    Term   31919   32371        +         0

>4539290       /111551

   len =     680   nex =       2

    Init   31692   31841        +         0
    Term   31919   32371        +         0

>4539290       /98650

   len =    1931   nex =       5

    Init   36398   36658        +         0
    Intr   36936   37160        +         0
    Intr   37542   37689        +         0
    Intr   37807   37886        +         0
    Term   37970   38328        +         0

>4539290       /6966

   len =     955   nex =       2

    Term   57171   56874        -         0
    Init   57828   57579        -         0

>4539290       /16673

   len =     976   nex =       1

    Sngl   57850   56875        -         0

>4539290       /31982

   len =    1005   nex =       2

    Term   57171   56874        -         0
    Init   57878   57579        -         0

>4539290       /37817

   len =    2214   nex =       8

    Init   58762   59031        +         0
    Intr   59151   59225        +         0
    Intr   59572   59628        +         0
    Intr   59712   59866        +         0
    Intr   59985   60119        +         0
    Intr   60211   60313        +         0
```

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```
     Intr    60402    60501          +         0
     Term    60611    60975          +         0

>4539290        /103513

   len =      198    nex =        1

     Sngl    60820    61017          +         0

>4539290        /10511

   len =     1487    nex =        5

     Term     4951     4724          -         0
     Intr     5226     5106          -         0
     Intr     5588     5309          -         0
     Intr     6021     5973          -         0
     Init     6210     6132          -         0

>4539290        /24045

   len =      713    nex =        2

     Init    86190    86240          +         0
     Term    86397    86784          +         0

>4539309        /31833

   len =     1938    nex =        3

     Init    23367    23550          +         0
     Intr    24243    24528          +         0
     Term    24866    25304          +         0

>4539309        /40069

   len =     1415    nex =        1

     Sngl    28842    30256          +         0

>4539309        /119970

   len =     1487    nex =        6

     Term    40137    39707          -         0
     Intr    40406    40272          -         0
     Intr    40622    40484          -         0
     Intr    40793    40703          -         0
     Intr    41003    40894          -         0
     Init    41193    41096          -         0

>4539309        /6351

   len =     2193    nex =        7

     Term    40137    39693          -         0
     Intr    40406    40272          -         0
     Intr    40622    40484          -         0
     Intr    40793    40703          -         0
     Intr    41003    40894          -         0
```

```
        Intr   41365   41096          -        0
        Init   41885   41628          -        0

   >4539309        /42753

       len =      911    nex =       3

        Term   65462   65294          -        0
        Intr   65761   65543          -        0
        Init   66204   66078          -        0

   >4539309        /18408

       len =     1111    nex =       2

        Term   65761   65543          -        0
        Init   66377   66078          -        0

   >4539309        /28021

       len =     1427    nex =       2

        Term   65761   65543          -        0
        Init   66693   66078          -        0

   >4539309        /6045

       len =      262    nex =       1

        Sngl   92055   91794          -        0

   >4539309        /8278

       len =     2297    nex =       5

        Term   94508   94131          -        0
        Intr   94898   94766          -        0
        Intr   95549   95481          -        0
        Intr   96124   95779          -        0
        Init   96427   96207          -        0

   >4539331        /38915

       len =      790    nex =       3

        Term   47037   46932          -        0
        Intr   47423   47129          -        0
        Init   47720   47526          -        0

   >4539353        /7769

       len =      556    nex =       1

        Sngl    2681    2129          -        0

   >4539353        /92155

       len =      411    nex =       1

        Sngl    2685    2275          -        0
```

```
>4539353        /1732

   len =     503   nex =       1

     Sngl  53207   53709          +        0

>4539378        /2917

   len =     370   nex =       1

     Sngl  34714   34349          -        0

>4539378        /2359

   len =    1179   nex =       5

     Init  61039   61098          +        0
     Intr  61178   61285          +        0
     Intr  61443   61485          +        0
     Intr  61581   61776          +        0
     Term  61866   62217          +        0

>4539378        /38408

   len =    1035   nex =       2

     Term  72291   71647          -        0
     Init  72681   72375          -        0

>4539378        /7322

   len =    1615   nex =       2

     Term  79437   78787          -        0
     Init  80401   79977          -        0

>4539402        /8976

   len =    2875   nex =      10

     Term  27509   27384          -        0
     Intr  27711   27643          -        0
     Intr  28151   28065          -        0
     Intr  28346   28239          -        0
     Intr  28516   28426          -        0
     Intr  29461   29382          -        0
     Intr  29655   29551          -        0
     Intr  29831   29737          -        0
     Intr  29988   29910          -        0
     Init  30258   30076          -        0

>4539402        /41359

   len =    3130   nex =      10

     Term  27509   27214          -        0
     Intr  27711   27643          -        0
     Intr  28151   28065          -        0
     Intr  28346   28239          -        0
```

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```
    Intr   28516   28426        -        0
    Intr   29461   29382        -        0
    Intr   29655   29551        -        0
    Intr   29831   29737        -        0
    Intr   29988   29910        -        0
    Init   30343   30076        -        0

>4539402        /41155

  len =     918    nex =       2

    Term   35555   35468        -        0
    Init   36257   36135        -        0

>4539402        /21228

  len =    1469    nex =       3

    Term   35555   35325        -        0
    Intr   36257   36135        -        0
    Init   36793   36352        -        0

>4539415        /742

  len =    1403    nex =       3

    Init   26047   26138        +        0
    Intr   26513   26661        +        0
    Term   27042   27449        +        0

>4539415        /14013

  len =     828    nex =       1

    Sngl   30384   30766        +        0

>4539415        /14312

  len =    2186    nex =       6

    Term   31374   31324        -        0
    Intr   31712   31634        -        0
    Intr   32178   32124        -        0
    Intr   32659   32576        -        0
    Intr   32806   32745        -        0
    Init   33072   32898        -        0

>4539448        /38976

  len =     401    nex =       1

    Sngl   19914   19514        -        0

>4539448        /13796

  len =    1156    nex =       2

    Term   19237   18760        -        0
    Init   19915   19490        -        0
```

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```
>4539448        /8846

    len =     1419    nex =        6

     Term     3827     3621          -         0
     Intr     4092     3925          -         0
     Intr     4400     4282          -         0
     Intr     4556     4495          -         0
     Intr     4723     4647          -         0
     Init     5039     4865          -         0

>4539448        /26831

    len =     1511    nex =        6

     Term     3827     3616          -         0
     Intr     4092     3925          -         0
     Intr     4400     4282          -         0
     Intr     4556     4495          -         0
     Intr     4723     4647          -         0
     Init     5126     4865          -         0

>4544365        /32729

    len =     1218    nex =        1

     Sngl    13401    14618          +         0

>4544365        /3839

    len =     2110    nex =        3

     Term    23021    22565          -         0
     Intr    23867    23630          -         0
     Init    24665    24122          -         0

>4544365        /32380

    len =     1118    nex =        2

     Term    44571    44182          -         0
     Init    45299    44761          -         0

>4544365        /28318

    len =      970    nex =        2

     Init    47075    47349          +         0
     Term    47442    48044          +         0

>4544365        /9657

    len =      533    nex =        1

     Sngl    80944    80412          -         0

>4544381        /30607

    len =     1011    nex =        3
```

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```
         Init    43399    43605          +         0
         Intr    43651    43747          +         0
         Term    43844    44402          +         0

    >4544381         /38904

       len =      207    nex =        1

         Sngl    44129    44335          +         0

    >4544381         /6922

       len =      827    nex =        1

         Sngl    93744    92918          -         0

    >4544381         /43076

       len =     1855    nex =        1

         Sngl    94771    92917          -         0

    >4544405         /3366

       len =     1270    nex =        6

         Init   108883   109010          +         0
         Intr   109298   109331          +         0
         Intr   109412   109584          +         0
         Intr   109671   109722          +         0
         Intr   109823   109890          +         0
         Term   109982   110152          +         0

    >4544405         /21909

       len =     1781    nex =        6

         Init    25216    25418          +         0
         Intr    25776    25847          +         0
         Intr    26206    26284          +         0
         Intr    26395    26527          +         0
         Intr    26623    26661          +         0
         Term    26747    26996          +         0

    >4544405         /11114

       len =     1439    nex =        1

         Sngl    51750    50803          -         0

    >4544405         /40781

       len =     1633    nex =        6

         Term    80918    80716          -         0
         Intr    81127    81067          -         0
         Intr    81666    81595          -         0
         Intr    81789    81740          -         0
         Intr    82070    81958          -         0
         Init    82348    82159          -         0
```

```
>4544405        /104918

   len =    1161   nex =       1

    Sngl   90572   90694        +        0

>4544435        /20723

   len =    1413   nex =       1

    Sngl   29535   30947        +        0

>4544435        /111553

   len =     510   nex =       1

    Sngl   30533   31042        +        0

>4544435        /117955

   len =    1450   nex =       3

    Init    3407    3589        +        0
    Intr    4304    4489        +        0
    Term    4602    4848        +        0

>4544435        /32573

   len =    3571   nex =       5

    Term   49696   49307        -        0
    Intr   50351   50080        -        0
    Intr   50691   50599        -        0
    Intr   52326   52225        -        0
    Init   52877   52427        -        0

>4544435        /38277

   len =     535   nex =       1

    Sngl   78881   78813        -        0

>4544435        /26961

   len =    1350   nex =       5

    Init   92102   92249        +        0
    Intr   92397   92496        +        0
    Intr   92587   92644        +        0
    Intr   92722   93078        +        0
    Term   93165   93451        +        0

>4544435        /10312

   len =    2925   nex =       6

    Term   94666   93767        -        0
    Intr   94855   94772        -        0
    Intr   94981   94933        -        0
```

```
     Intr   95346   95304        -       0
     Intr   95580   95523        -       0
     Init   96143   96015        -       0

>4557056       /38982

    len =    1316   nex =       4

     Term   13849   13681        -       0
     Intr   14205   14054        -       0
     Intr   14347   14277        -       0
     Init   14637   14454        -       0

>4557061       /7520

    len =    1480   nex =       1

     Sngl   23980   24135        +       0

>4557061       /39407

    len =    1630   nex =       3

     Term   26056   25532        -       0
     Intr   26195   26155        -       0
     Init   27156   26894        -       0

>4557061       /5435

    len =    1632   nex =       3

     Term   26056   25532        -       0
     Intr   26195   26155        -       0
     Init   27012   26894        -       0

>4558521       /23664

    len =     898   nex =       1

     Sngl   30777   31674        +       0

>4558521       /37480

    len =     760   nex =       2

     Term   41994   41798        -       0
     Init   42557   42246        -       0

>4558521       /27813

    len =    1810   nex =       4

     Term   60751   60244        -       0
     Intr   61451   61243        -       0
     Intr   61676   61576        -       0
     Init   62053   61830        -       0

>4558521       /7488

    len =    1414   nex =       2
```

```
     Term   83600   83130         -        0
     Init   84090   83849         -        0

>4558586       /18981

    len =    3812   nex =      12

     Term    7302    6978         -        0
     Intr    7549    7499         -        0
     Intr    7962    7870         -        0
     Intr    8232    8157         -        0
     Intr    8456    8340         -        0
     Intr    8680    8616         -        0
     Intr    9112    8999         -        0
     Intr    9310    9212         -        0
     Intr    9563    9472         -        0
     Intr   10014    9891         -        0
     Intr   10285   10118         -        0
     Init   10789   10619         -        0

>4558586       /18724

    len =    4210   nex =      14

     Term   11491   11046         -        0
     Intr   11945   11761         -        0
     Intr   12171   12058         -        0
     Intr   12446   12359         -        0
     Intr   12612   12540         -        0
     Intr   13246   13121         -        0
     Intr   13516   13427         -        0
     Intr   13708   13598         -        0
     Intr   14034   13801         -        0
     Intr   14205   14128         -        0
     Intr   14379   14308         -        0
     Intr   14551   14468         -        0
     Intr   14778   14659         -        0
     Init   15254   15074         -        0

>4558586       /2070

    len =    1824   nex =       7

     Term   33890   33632         -        0
     Intr   34060   33981         -        0
     Intr   34417   34262         -        0
     Intr   34645   34510         -        0
     Intr   34881   34730         -        0
     Intr   35164   35075         -        0
     Init   35455   35254         -        0

>4558586       /157873

    len =     649   nex =       3

     Term   34881   34807         -        0
     Intr   35164   35075         -        0
     Init   35455   35254         -        0
```

```
>4558586       /35814

   len =    1938   nex =       7

    Term   33890   33575        -         0
    Intr   34060   33981        -         0
    Intr   34417   34262        -         0
    Intr   34645   34510        -         0
    Intr   34881   34730        -         0
    Intr   35164   35075        -         0
    Init   35512   35254        -         0

>4558586       /19277

   len =     655   nex =       1

    Sngl   77232   77886        +         0

>4558586       /106135

   len =     635   nex =       1

    Sngl   84401   85035        +         0

>4558590       /41858

   len =    2113   nex =       3

    Init    3306    3445        +         0
    Intr    3564    4886        +         0
    Term    5194    5418        +         0

>4558590       /14128

   len =    1425   nex =       2

    Init    3948    4886        +         0
    Term    5194    5372        +         0

>4558656       /95747

   len =     859   nex =       1

    Sngl   82575   83433        +         0

>4558656       /25241

   len =     657   nex =       1

    Sngl   82776   83432        +         0

>4558656       /17483

   len =    2449   nex =       2

    Term   98045   96936        -         0
    Init   99384   98603        -         0

>4558674       /110217
```

```
    len =      430    nex =        1

      Sngl   49557    49135          -         0

>4558674          /29241

    len =      730    nex =        1

      Sngl   50473    51194          +         0

>4558674          /33853

    len =      550    nex =        1

      Sngl   50532    51077          +         0

>4559319          /19749

    len =     2077    nex =        6

      Init   51859    52123          +         0
      Intr   52351    52657          +         0
      Intr   52735    52822          +         0
      Intr   52920    53029          +         0
      Intr   53123    53271          +         0
      Term   53559    53620          +         0

>4559319          /17752

    len =     2650    nex =        5

      Init   65746    66206          +         0
      Intr   66784    66955          +         0
      Intr   67501    67676          +         0
      Intr   67761    67919          +         0
      Term   68034    68387          +         0

>4559319          /19221

    len =      850    nex =        2

      Init   75881    76149          +         0
      Term   76407    76724          +         0

>4559319          /104159

    len =      523    nex =        1

      Sngl   77455    76942          -         0

>4559319          /41181

    len =     3370    nex =        6

      Term   77618    77108          -         0
      Intr   77969    77856          -         0
      Intr   78281    78114          -         0
      Intr   79196    78954          -         0
      Intr   79602    79519          -         0
      Init   80468    79689          -         0
```

```
>4559319        /37057

   len =     3250    nex =       12

     Init   84481   84692         +         0
     Intr   85229   85264         +         0
     Intr   85302   85358         +         0
     Intr   85490   85573         +         0
     Intr   85652   85723         +         0
     Intr   85920   85991         +         0
     Intr   86232   86272         +         0
     Intr   86584   86650         +         0
     Intr   86992   87183         +         0
     Intr   87283   87372         +         0
     Intr   87461   87568         +         0
     Term   87662   87727         +         0

>4559344        /17434

   len =     1227    nex =        2

     Term   99351   98868         -         0
     Init  100094   99455         -         0

>4559344        /30071

   len =     1657    nex =        5

     Term   36166   35586         -         0
     Intr   36370   36254         -         0
     Intr   36762   36454         -         0
     Intr   37029   36967         -         0
     Init   37242   37124         -         0

>4559344        /10798

   len =     1189    nex =        5

     Init   45685   45867         +         0
     Intr   45942   46037         +         0
     Intr   46237   46359         +         0
     Intr   46569   46608         +         0
     Term   46704   46873         +         0

>4559344        /1916

   len =      490    nex =        1

     Sngl   57577   57090         -         0

>4559344        /6722

   len =      409    nex =        1

     Sngl   57584   57176         -         0

>4559344        /10261

   len =      522    nex =        1
```

```
    Sngl   57598   57077         -        0

>4559344       /97914

   len =     1291   nex =       3

    Init   78733   78958         +        0
    Intr   79545   79694         +        0
    Term   79797   80023         +        0

>4559344       /14570

   len =     2280   nex =       7

    Init   81776   82132         +        0
    Intr   82307   82649         +        0
    Intr   82774   82992         +        0
    Intr   83092   83151         +        0
    Intr   83231   83386         +        0
    Intr   83500   83593         +        0
    Term   83750   84055         +        0

>4559344       /122569

   len =      214   nex =       1

    Sngl   83842   84055         +        0

>4559344       /36270

   len =     1377   nex =       1

    Sngl   91284   90056         -        0

>4559344       /29774

   len =     1937   nex =       3

    Term   91284   90056         -        0
    Intr   91468   91366         -        0
    Init   91992   91560         -        0

>4559375       /102981

   len =      568   nex =       1

    Sngl   19716   20275         +        0

>4559375       /94104

   len =      516   nex =       1

    Sngl   21573   22085         +        0

>4559375       /12474

   len =      737   nex =       1

    Sngl   26746   26629         -        0
```

```
>4559375       /35220

   len =    1760   nex =       7

    Init   38581   38611        +        0
    Intr   38834   39022        +        0
    Intr   39074   39179        +        0
    Intr   39265   39450        +        0
    Intr   39547   39712        +        0
    Intr   39807   40123        +        0
    Term   40251   40340        +        0

>4567193       /38340

   len =    1426   nex =       4

    Term   16701   16432        -        0
    Intr   17094   16777        -        0
    Intr   17731   17187        -        0
    Init   17857   17801        -        0

>4567193       /17172

   len =    1760   nex =       6

    Term   28035   27578        -        0
    Intr   28415   28113        -        0
    Intr   28602   28501        -        0
    Intr   28796   28685        -        0
    Intr   29087   29011        -        0
    Init   29337   29173        -        0

>4567193       /206273

   len =    2060   nex =       7

    Term   30185   29858        -        0
    Intr   30460   30268        -        0
    Intr   30657   30541        -        0
    Intr   31111   30896        -        0
    Intr   31336   31225        -        0
    Intr   31570   31506        -        0
    Init   31737   31656        -        0

>4567193       /15405

   len =     192   nex =       1

    Sngl   39059   39250        +        0

>4567193       /30185

   len =    1632   nex =       4

    Term   41869   41427        -        0
    Intr   42547   42403        -        0
    Intr   42721   42661        -        0
    Init   43058   42799        -        0
```

```
>4567193       /15081

   len =     928   nex =       2

    Init   65231   65374        +        0
    Term   65556   66158        +        0

>4567193       /153017

   len =     616   nex =       2

    Init   65262   65374        +        0
    Term   65556   65877        +        0

>4567237       /13309

   len =    1334   nex =       5

    Init   37681   37817        +        0
    Intr   38008   38054        +        0
    Intr   38602   38657        +        0
    Intr   38750   38866        +        0
    Term   38969   39006        +        0

>4567237       /25127

   len =    2314   nex =       8

    Init   52101   52260        +        0
    Intr   52608   52692        +        0
    Intr   52766   52834        +        0
    Intr   52909   53021        +        0
    Intr   53119   53230        +        0
    Intr   53328   53471        +        0
    Intr   53682   53856        +        0
    Term   54076   54414        +        0

>4567237       /22703

   len =    1450   nex =       3

    Term   56329   56148        -        0
    Intr   56617   56415        -        0
    Init   57116   56959        -        0

>4567237       /33370

   len =     685   nex =       3

    Term   76509   76290        -        0
    Intr   76701   76598        -        0
    Init   76968   76910        -        0

>4567237       /11284

   len =     693   nex =       3

    Term   76509   76285        -        0
    Intr   76701   76598        -        0
    Init   76968   76910        -        0
```

```
>4567259       /33

   len =    1944   nex =       6

    Term     678     284        -        0
    Intr     980     761        -        0
    Intr    1195    1063        -        0
    Intr    1445    1328        -        0
    Intr    1621    1527        -        0
    Init    2227    2024        -        0

>4567259       /38112

   len =    1572   nex =       5

    Term   26086   25917        -        0
    Intr   26263   26173        -        0
    Intr   26465   26356        -        0
    Intr   26929   26660        -        0
    Init   27488   27013        -        0

>4567259       /20834

   len =    3815   nex =      14

    Init   29608   30133        +        0
    Intr   30762   30856        +        0
    Intr   30987   31095        +        0
    Intr   31188   31234        +        0
    Intr   31412   31467        +        0
    Intr   31629   31767        +        0
    Intr   31884   32021        +        0
    Intr   32100   32168        +        0
    Intr   32254   32364        +        0
    Intr   32467   32595        +        0
    Intr   32688   32765        +        0
    Intr   32854   32925        +        0
    Intr   33026   33088        +        0
    Term   33177   33422        +        0

>4567259       /121118

   len =    2034   nex =       9

    Init   31629   31767        +        0
    Intr   31884   32021        +        0
    Intr   32100   32168        +        0
    Intr   32254   32364        +        0
    Intr   32467   32595        +        0
    Intr   32688   32765        +        0
    Intr   32854   32925        +        0
    Intr   33026   33088        +        0
    Term   33177   33474        +        0

>4567259       /21730

   len =    1774   nex =       6

    Term    2963    2647        -        0
```

```
     Intr    3263    3044         -         0
     Intr    3478    3346         -         0
     Intr    3746    3629         -         0
     Intr    3925    3831         -         0
     Init    4420    4216         -         0

>4567259        /34470

    len =    2606   nex =        8

     Init    5119    5168         +         0
     Intr    5278    5355         +         0
     Intr    5522    5546         +         0
     Intr    5632    5664         +         0
     Intr    5971    6101         +         0
     Intr    6291    6511         +         0
     Intr    6606    6853         +         0
     Term    6954    7195         +         0

>4567259        /33121

    len =    2209   nex =        9

     Init   46682   46836         +         0
     Intr   47131   47236         +         0
     Intr   47341   47382         +         0
     Intr   47532   47597         +         0
     Intr   47686   47802         +         0
     Intr   47939   48214         +         0
     Intr   48303   48350         +         0
     Intr   48427   48548         +         0
     Term   48699   48890         +         0

>4567259        /36598

    len =    2212   nex =       11

     Init   46682   46836         +         0
     Intr   46924   47015         +         0
     Intr   47131   47236         +         0
     Intr   47341   47382         +         0
     Intr   47532   47597         +         0
     Intr   47686   47802         +         0
     Intr   47939   48013         +         0
     Intr   48112   48214         +         0
     Intr   48303   48350         +         0
     Intr   48427   48548         +         0
     Term   48699   48893         +         0

>4567259        /109948

    len =     475   nex =        2

     Init   48460   48548         +         0
     Term   48699   48934         +         0

>4567259        /1285

    len =    1185   nex =        1
```

09955325.061601

```
    Sngl   52746   53930         +        0

>4567259        /21453

  len =    1410   nex =        3

    Term   69518   68949         -        0
    Intr   69807   69688         -        0
    Init   70358   69891         -        0

>4567259        /33656

  len =     463   nex =        1

    Sngl    9884   10346         +        0

>4567259        /156111

  len =     322   nex =        1

    Sngl    9910   10231         +        0

>4567300        /125386

  len =     910   nex =        3

    Init   13167   13335         +        0
    Intr   13426   13542         +        0
    Term   13632   14051         +        0

>4567300        /101253

  len =     859   nex =        3

    Init   33481   33676         +        0
    Intr   33813   33938         +        0
    Term   34031   34339         +        0

>4567300        /123234

  len =    1330   nex =        4

    Term   34776   34516         -        0
    Intr   35061   34867         -        0
    Intr   35283   35188         -        0
    Init   35576   35463         -        0

>4567300        /24221

  len =     393   nex =        1

    Sngl    8449    8841         +        0

>4572664        /2867

  len =    1510   nex =        4

    Term   55509   55260         -        0
    Intr   55693   55605         -        0
    Intr   55882   55783         -        0
```

09855326.051701

```
     Init    56077   56005          -         0

>4572664        /34807

     len =     1418    nex =        1

     Sngl    59430   60847          +         0

>4572664        /113985

     len =      173    nex =        1

     Sngl    66412   66584          +         0

>4572664        /11953

     len =     1510    nex =        3

     Init    66481   67144          +         0
     Intr    67636   67764          +         0
     Term    67855   67987          +         0

>4572664        /34681

     len =     1379    nex =        2

     Term    73338   72795          -         0
     Init    74173   73545          -         0

>4572664        /37401

     len =     1316    nex =        2

     Init    76483   76979          +         0
     Term    77234   77798          +         0

>4572664        /36719

     len =     1231    nex =        1

     Sngl    79365   78135          -         0

>4580365        /27596

     len =     1365    nex =        4

     Term    99273   99000          -         0
     Intr    99825   99644          -         0
     Intr   100012   99954          -         0
     Init   100364  100196          -         0

>4580365        /27081

     len =      492    nex =        1

     Sngl    43711   43220          -         0

>4580365        /39571

     len =     2540    nex =        9
```

09595326.061600

```
      Init   60690   60861        +        0
      Intr   61125   61240        +        0
      Intr   61348   61407        +        0
      Intr   61645   61830        +        0
      Intr   61925   62029        +        0
      Intr   62147   62243        +        0
      Intr   62438   62545        +        0
      Intr   62686   62821        +        0
      Term   62943   63229        +        0

>4580365       /108216

     len =    1090   nex =       4

      Init   62147   62243        +        0
      Intr   62438   62545        +        0
      Intr   62686   62821        +        0
      Term   62943   63229        +        0
>4580365       /158765

     len =    2230   nex =       4

      Init   96491   97832        +        0
      Intr   97943   98008        +        0
      Intr   98083   98148        +        0
      Term   98215   98259        +        0

>4580365       /3900

     len =    1249   nex =       5

      Init   97546   97832        +        0
      Intr   97943   98008        +        0
      Intr   98083   98148        +        0
      Intr   98215   98259        +        0
      Term   98386   98794        +        0

>4580454       /36815

     len =    2248   nex =       6

      Term   23978   23775        -        0
      Intr   24212   24072        -        0
      Intr   24520   24304        -        0
      Intr   24794   24625        -        0
      Intr   25442   25197        -        0
      Init   26022   25523        -        0

>4580454       /12246

     len =    1961   nex =       7

      Init   26312   26626        +        0
      Intr   26975   27027        +        0
      Intr   27104   27213        +        0
      Intr   27296   27463        +        0
      Intr   27553   27650        +        0
      Intr   27792   27918        +        0
      Term   28033   28272        +        0
```

09559326.061600

```
>4580454        /123469

    len =     2112    nex =       7

     Init   29262   29569         +         0
     Intr   30006   30058         +         0
     Intr   30153   30262         +         0
     Intr   30351   30518         +         0
     Intr   30608   30705         +         0
     Intr   30832   30958         +         0
     Term   31073   31373         +         0

>4580732        /43073

    len =     1642    nex =       1

     Sngl  102136  103777         +         0

>4580732        /1069

    len =     1112    nex =       3

     Term   42616   42417         -         0
     Intr   43115   43025         -         0
     Init   43528   43375         -         0

>4580744        /34680

    len =     2025    nex =       4

     Term   15210   14726         -         0
     Intr   15766   15298         -         0
     Intr   16116   15998         -         0
     Init   16750   16441         -         0

>4580744        /1294

    len =     2050    nex =       5

     Term   22434   22008         -         0
     Intr   22792   22540         -         0
     Intr   23073   22873         -         0
     Intr   23265   23147         -         0
     Init   24057   23791         -         0

>4580744        /6124

    len =      251    nex =       1

     Sngl   31321   31071         -         0

>4580744        /36505

    len =     3231    nex =       1

     Sngl   32321   31071         -         0

>4580744        /97415
```

```
    len =      854    nex =        1

     Sngl    40155    39760          -        0

>4580744          /117519

    len =     1292    nex =        1

     Sngl    41250    42541          +        0

>4580744          /783

    len =     1575    nex =        5

     Term    44523    44000          -        0
     Intr    44831    44655          -        0
     Intr    45013    44928          -        0
     Intr    45231    45112          -        0
     Init    45559    45529          -        0

>4580744          /37712

    len =     2875    nex =       12

     Term    44523    44251          -        0
     Intr    44831    44655          -        0
     Intr    45013    44928          -        0
     Intr    45231    45112          -        0
     Intr    45559    45529          -        0
     Intr    45722    45647          -        0
     Intr    45995    45908          -        0
     Intr    46153    46092          -        0
     Intr    46314    46238          -        0
     Intr    46521    46419          -        0
     Intr    46677    46616          -        0
     Init    47125    47027          -        0

>4580744          /37965

    len =     3581    nex =       14

     Term    55823    55611          -        0
     Intr    56063    55914          -        0
     Intr    56251    56154          -        0
     Intr    56471    56331          -        0
     Intr    56734    56585          -        0
     Intr    56994    56818          -        0
     Intr    57262    57082          -        0
     Intr    57604    57559          -        0
     Intr    57984    57921          -        0
     Intr    58092    58070          -        0
     Intr    58311    58239          -        0
     Intr    58627    58496          -        0
     Intr    58799    58713          -        0
     Init    59191    59053          -        0

>4580744          /40575

    len =     3683    nex =       14
```

09595326.061600

```
        Term   55823   55622        -         0
        Intr   56063   55914        -         0
        Intr   56251   56154        -         0
        Intr   56471   56331        -         0
        Intr   56734   56585        -         0
        Intr   56994   56818        -         0
        Intr   57262   57082        -         0
        Intr   57604   57559        -         0
        Intr   57984   57921        -         0
        Intr   58092   58070        -         0
        Intr   58311   58239        -         0
        Intr   58627   58496        -         0
        Intr   58799   58713        -         0
        Init   59304   59053        -         0

>4580744       /28624

      len =    2278    nex =       6

        Init   60438   60505        +         0
        Intr   60760   60832        +         0
        Intr   61116   61231        +         0
        Intr   61311   61403        +         0
        Intr   61511   61610        +         0
        Term   61781   62029        +         0

>4580744       /94743

      len =     463    nex =       2

        Init   67354   67484        +         0
        Term   67496   67816        +         0

>4580744       /39230

      len =     920    nex =       2

        Term   74699   74303        -         0
        Init   75222   74942        -         0

>4580744       /18908

      len =    2052    nex =       6

        Init   75765   76184        +         0
        Intr   76412   76510        +         0
        Intr   76599   76760        +         0
        Intr   76852   76973        +         0
        Intr   77156   77275        +         0
        Term   77360   77816        +         0

>4580745       /2757

      len =     777    nex =       1

        Sngl   11301   12077        +         0

>4580745       /32177

      len =     757    nex =       1
```

00555325.061500

```
     Sngl   11324   12080          +         0

>4580745        /159018

   len =      694    nex =        1

     Sngl   11389   12082          +         0

>4580745        /3297

   len =     2687    nex =       10

     Term   14071   13559          -         0
     Intr   14202   14142          -         0
     Intr   14379   14272          -         0
     Intr   14542   14483          -         0
     Intr   14703   14625          -         0
     Intr   14942   14872          -         0
     Intr   15083   15027          -         0
     Intr   15381   15201          -         0
     Intr   15714   15550          -         0
     Init   16245   16004          -         0

>4580745        /12622

   len =     2315    nex =        6

     Init   59279   59398          +         0
     Intr   59843   59965          +         0
     Intr   60132   60197          +         0
     Intr   60370   60435          +         0
     Intr   60831   60911          +         0
     Term   61005   61589          +         0

>4580745        /10117

   len =     2193    nex =        6

     Term    7149    6887          -         0
     Intr    7300    7240          -         0
     Intr    7562    7493          -         0
     Intr    7945    7813          -         0
     Intr    8854    8764          -         0
     Init    9079    8961          -         0

>4581084        /32373

   len =     1583    nex =        4

     Term   13458   13179          -         0
     Intr   13805   13635          -         0
     Intr   14191   14006          -         0
     Init   14761   14500          -         0

>4581084        /3173

   len =     2123    nex =        5

     Init   14954   15412          +         0
```

```
      Intr   15767   16020          +        0
      Intr   16168   16291          +        0
      Intr   16405   16509          +        0
      Term   16791   17076          +        0

>4581084       /34830

   len =    1601   nex =        3

      Term   22349   21931          -        0
      Intr   23000   22548          -        0
      Init   23531   23196          -        0

>4581084       /28597

   len =    1825   nex =        2

      Term   40647   39196          -        0
      Init   41020   40887          -        0

>4581084       /41311

   len =    2171   nex =        7

      Init   41924   42481          +        0
      Intr   42756   42849          +        0
      Intr   42930   42963          +        0
      Intr   43048   43102          +        0
      Intr   43184   43267          +        0
      Intr   43366   43653          +        0
      Term   43750   44094          +        0

>4581084       /37650

   len =    2337   nex =        8

      Term    3055    2967          -        0
      Intr    3229    3167          -        0
      Intr    3411    3322          -        0
      Intr    3664    3604          -        0
      Intr    3843    3749          -        0
      Intr    4154    4065          -        0
      Intr    4317    4231          -        0
      Init    4792    4411          -        0

>4581084       /17428

   len =    2451   nex =        7

      Term    3055    2967          -        0
      Intr    3229    3167          -        0
      Intr    3411    3322          -        0
      Intr    3664    3604          -        0
      Intr    3843    3749          -        0
      Intr    4154    4065          -        0
      Init    4317    4231          -        0

>4581084       /93110

   len =    1289   nex =        3
```

```
      Term    60581   60181         -         0
      Intr    61120   61028         -         0
      Init    61458   61210         -         0

>4581084        /38429

    len =     2273   nex =        6

      Term    60581   60221         -         0
      Intr    61120   61028         -         0
      Intr    61458   61210         -         0
      Intr    61709   61557         -         0
      Intr    62108   61784         -         0
      Init    62493   62191         -         0

>4581084        /5677

    len =      943   nex =        2

      Init    72815   73073         +         0
      Term    73160   73757         +         0

>4581103        /36296

    len =     1853   nex =        4

      Init   105971  106313         +         0
      Intr   106543  106838         +         0
      Intr   107212  107352         +         0
      Term   107448  107823         +         0

>4581103        /119868

    len =      638   nex =        2

      Term    25527   25372         -         0
      Init    26009   25647         -         0

>4581103        /26940

    len =     1076   nex =        3

      Init    49009   49124         +         0
      Intr    49202   49461         +         0
      Term    49704   50084         +         0

>4581103        /23995

    len =      755   nex =        1

      Sngl     5195    5949         +         0

>4581103        /31917

    len =     2086   nex =        3

      Init    52571   52873         +         0
      Intr    54100   54217         +         0
      Term    54298   54656         +         0
```

```
>4581103       /27679

   len =     1056   nex =        4

     Init   72192   72336        +        0
     Intr   72438   72565        +        0
     Intr   72751   72855        +        0
     Term   72981   73247        +        0

>4581103       /40637

   len =     2148   nex =        6

     Term   86242   85852        -        0
     Intr   86439   86344        -        0
     Intr   86717   86593        -        0
     Intr   87013   86870        -        0
     Intr   87427   87104        -        0
     Init   87999   87653        -        0

>4581103       /19417

   len =     1281   nex =        4

     Term   88901   88344        -        0
     Intr   89054   88975        -        0
     Intr   89191   89149        -        0
     Init   89329   89270        -        0

>4581103       /23735

   len =     1579   nex =        2

     Init   90125   90516        +        0
     Term   90763   91703        +        0

>4581138       /17193

   len =     2079   nex =        6

     Init   17050   17171        +        0
     Intr   17298   17403        +        0
     Intr   17518   17646        +        0
     Intr   17927   18071        +        0
     Intr   18203   18760        +        0
     Term   18845   19128        +        0

>4581138       /5234

   len =      812   nex =        2

     Init   18317   18760        +        0
     Term   18845   19128        +        0

>4581138       /13024

   len =     1680   nex =        3

     Term   46797   45819        -        0
```

```
    Intr   47110   46883        -        0
    Init   47498   47408        -        0

>4581138       /18705

  len =     550   nex =        1

    Sngl   52038   51498        -        0

>4581138       /39339

  len =     291   nex =        1

    Sngl   70179   69889        -        0

>4581138       /9198

  len =     646   nex =        3

    Term   70209   69891        -        0
    Intr   70349   70293        -        0
    Init   70536   70438        -        0

>4581138       /12548

  len =    2972   nex =       12

    Term   70209   69940        -        0
    Intr   70349   70293        -        0
    Intr   70629   70438        -        0
    Intr   70921   70727        -        0
    Intr   71094   71017        -        0
    Intr   71319   71182        -        0
    Intr   71648   71466        -        0
    Intr   71860   71780        -        0
    Intr   72035   71949        -        0
    Intr   72187   72140        -        0
    Intr   72413   72285        -        0
    Init   72911   72769        -        0

>4581138       /12298

  len =    3528   nex =       16

    Init   88301   88493        +        0
    Intr   88550   88646        +        0
    Intr   88730   88827        +        0
    Intr   88922   89077        +        0
    Intr   89176   89253        +        0
    Intr   89364   89495        +        0
    Intr   89613   89693        +        0
    Intr   89822   89893        +        0
    Intr   89997   90086        +        0
    Intr   90230   90374        +        0
    Intr   90498   90586        +        0
    Intr   90705   90800        +        0
    Intr   90897   90964        +        0
    Intr   91053   91152        +        0
    Intr   91480   91539        +        0
    Term   91677   91828        +        0
```

```
>4581138        /20959

   len =    2279    nex =       7

    Term   92547    92107        -        0
    Intr   92826    92611        -        0
    Intr   93078    92954        -        0
    Intr   93363    93174        -        0
    Intr   93544    93450        -        0
    Intr   93830    93620        -        0
    Init   94385    94134        -        0

>4581161        /6542

   len =     974    nex =       2

    Term  103655   103129        -        0
    Init  104102   104014        -        0

>4581161        /4401

   len =     944    nex =       2

    Term  103655   103178        -        0
    Init  104121   104014        -        0

>4581161        /3929

   len =    2594    nex =       5

    Term  101826   101547        -        0
    Intr  102280   102202        -        0
    Intr  102758   102630        -        0
    Intr  103655   103281        -        0
    Init  104140   104014        -        0

>4581161        /207629

   len =    1078    nex =       1

    Sngl  104140   104014        -        0

>4581161        /153194

   len =    1076    nex =       2

    Term  103655   103065        -        0
    Init  104140   104014        -        0

>4581161        /113443

   len =    1014    nex =       1

    Sngl  104140   104014        -        0

>4581161        /105261

   len =    1012    nex =       2
```

```
     Term  103655  103129         -        0
     Init  104140  104014         -        0

>4581161       /23214

    len =     970   nex =       2

     Term  103655  103172         -        0
     Init  104140  104014         -        0

>4581161       /92991

    len =     970   nex =       2

     Term  103655  103178         -        0
     Init  104140  104014         -        0

>4581161       /109952

    len =     951   nex =       2

     Term  103655  103190         -        0
     Init  104140  104014         -        0

>4581161       /118540

    len =     597   nex =       2

     Term  103655  103544         -        0
     Init  104140  104014         -        0

>4581161       /18215

    len =    2507   nex =       1

     Sngl  104144  104014         -        0

>4581161       /24845

    len =     970   nex =       2

     Term  103655  103177         -        0
     Init  104144  104014         -        0

>4581161       /24667

    len =     970   nex =       2

     Term  103655  103178         -        0
     Init  104144  104014         -        0

>4581161       /3416

    len =    1815   nex =       5

     Init   12372   12490         +        0
     Intr   13293   13348         +        0
     Intr   13421   13473         +        0
     Intr   13699   13817         +        0
     Term   13921   14186         +        0
```

```
>4581161        /9243

  len =      943    nex =        2

   Init   12408    12490         +          0
   Term   13293    13350         +          0

>4581161        /1618

  len =      698    nex =        2

   Init   15445    15838         +          0
   Term   15921    16142         +          0

>4581161        /6509

  len =      395    nex =        1

   Sngl   80928    80534         -          0

>4581161        /12929

  len =      396    nex =        1

   Sngl   80929    80534         -          0

>4581161        /24190

  len =      933    nex =        2

   Term   81003    80534         -          0
   Init   81466    81341         -          0

>4581161        /39985

  len =      934    nex =        2

   Term   81003    80534         -          0
   Init   81467    81341         -          0

>4581161        /20104

  len =      911    nex =        2

   Term   81003    80557         -          0
   Init   81467    81341         -          0

>4581161        /109026

  len =      753    nex =        2

   Term   81003    80715         -          0
   Init   81467    81341         -          0

>4581161        /34326

  len =      938    nex =        2

   Term   81003    80534         -          0
```

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```
     Init   81471   81341        -        0

>4581161       /12459

   len =     915   nex =       2

     Term   81003   80557        -        0
     Init   81471   81341        -        0

>4581161       /103273

   len =     910   nex =       0

>4581161       /102088

   len =     671   nex =       2

     Term   81003   80801        -        0
     Init   81471   81341        -        0

>4582411       /33336

   len =    2810   nex =       7

     Init   45451   45474        +        0
     Intr   45798   45903        +        0
     Intr   46005   46073        +        0
     Intr   46212   46705        +        0
     Intr   47033   47294        +        0
     Intr   47380   47588        +        0
     Term   47685   47897        +        0

>4582411       /21223

   len =    2816   nex =       7

     Init   45451   45474        +        0
     Intr   45798   45903        +        0
     Intr   46005   46073        +        0
     Intr   46212   46705        +        0
     Intr   47033   47294        +        0
     Intr   47380   47588        +        0
     Term   47685   47903        +        0

>4582411       /12397

   len =    1719   nex =       5

     Init   48106   48233        +        0
     Intr   48506   48720        +        0
     Intr   49001   49081        +        0
     Intr   49174   49521        +        0
     Term   49615   49824        +        0

>4582428       /13146

   len =    1111   nex =       2

     Init   14118   14404        +        0
     Term   14774   15228        +        0
```

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```
>4582428       /16737

   len =    1407   nex =       5

     Init   18518   18583        +        0
     Intr   18928   19009        +        0
     Intr   19112   19183        +        0
     Intr   19501   19553        +        0
     Term   19701   19924        +        0

>4582428       /97480

   len =    1161   nex =       4

     Init   18928   19009        +        0
     Intr   19112   19183        +        0
     Intr   19501   19553        +        0
     Term   19701   19911        +        0

>4582437       /2900

   len =     397   nex =       1

     Sngl   19712   19316        -        0

>4582437       /99899

   len =     680   nex =       1

     Sngl    7826    8505        +        0

>4582444       /103070

   len =     790   nex =       2

     Init    2508    3031        +        0
     Term    3115    3290        +        0

>4582444       /37180

   len =    1440   nex =       1

     Sngl   25314   26753        +        0

>4582444       /123564

   len =    1450   nex =       1

     Sngl   25977   26038        +        0

>4582444       /25550

   len =    1675   nex =       5

     Term   27247   27080        -        0
     Intr   27553   27435        -        0
     Intr   27722   27661        -        0
     Intr   28224   28148        -        0
     Init   28606   28475        -        0
```

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```
>4582444        /19236

   len =     651   nex =       1

     Sngl   34810   35460         +        0

>4582444        /9568

   len =    1311   nex =       5

     Init   39087   39157         +        0
     Intr   39241   39388         +        0
     Intr   39469   39640         +        0
     Intr   39922   40108         +        0
     Term   40198   40397         +        0

>4582444        /15190

   len =     718   nex =       2

     Init   39945   40108         +        0
     Term   40198   40662         +        0

>4582444        /15416

   len =    1431   nex =       2

     Term   62850   62359         -        0
     Init   63789   63368         -        0

>4582444        /74

   len =    1311   nex =       1

     Sngl   71021   70539         -        0

>4582444        /25159

   len =    1106   nex =       3

     Term   75373   75084         -        0
     Intr   75585   75448         -        0
     Init   76189   76007         -        0

>4582444        /94470

   len =     430   nex =       1

     Sngl   78400   77979         -        0

>4584339        /13257

   len =    2291   nex =       6

     Term   19077   18435         -        0
     Intr   19277   19154         -        0
     Intr   19501   19359         -        0
     Intr   19724   19589         -        0
     Intr   20156   19825         -        0
```

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```
    Init   20725   20474          -         0

>4584339        /641

   len =    1150   nex =        1

    Sngl   26683   25535          -         0

>4584339        /552

   len =    1065   nex =        2

    Init   28913   29192          +         0
    Term   29589   29977          +         0

>4584351        /17506

   len =     490   nex =        1

    Sngl   30155   29673          -         0

>4584351        /41808

   len =     521   nex =        1

    Sngl   30170   30061          -         0

>4584351        /143435

   len =     471   nex =        1

    Sngl   30170   29700          -         0

>4584351        /14272

   len =     575   nex =        1

    Sngl   30249   29675          -         0

>4584351        /91878

   len =    1832   nex =        5

    Term   30771   30505          -         0
    Intr   31297   31037          -         0
    Intr   31481   31377          -         0
    Intr   31721   31591          -         0
    Init   32336   32137          -         0

>4584387        /7818

   len =    1390   nex =        1

    Sngl   19033   19118          +         0

>4584387        /12275

   len =     490   nex =        1

    Sngl   31615   31127          -         0
```

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```
>4584387       /18912

  len =    5325   nex =      20

   Init   31825   31912        +        0
   Intr   32018   32165        +        0
   Intr   32301   32493        +        0
   Intr   32573   32662        +        0
   Intr   32903   32965        +        0
   Intr   33174   33243        +        0
   Intr   33394   33458        +        0
   Intr   33795   33849        +        0
   Intr   33973   34094        +        0
   Intr   34178   34293        +        0
   Intr   34678   34747        +        0
   Intr   34996   35100        +        0
   Intr   35202   35291        +        0
   Intr   35374   35522        +        0
   Intr   35706   35817        +        0
   Intr   35900   35983        +        0
   Intr   36106   36186        +        0
   Intr   36275   36499        +        0
   Intr   36584   36710        +        0
   Term   36857   37134        +        0

>4584387       /6665

  len =    2598   nex =       7

   Init   38193   38274        +        0
   Intr   38429   38504        +        0
   Intr   38670   38833        +        0
   Intr   38917   39067        +        0
   Intr   39199   39321        +        0
   Intr   39439   39534        +        0
   Term   39625   40136        +        0

>4584519       /33414

  len =    2899   nex =      10

   Term   97811   97456        -        0
   Intr   98170   97906        -        0
   Intr   98475   98309        -        0
   Intr   98711   98568        -        0
   Intr   99028   98919        -        0
   Intr   99221   99113        -        0
   Intr   99408   99319        -        0
   Intr   99531   99493        -        0
   Intr   99800   99606        -        0
   Init   99974   99924        -        0

>4584519       /21149

  len =    1545   nex =       7

   Term  100825  100655        -        0
   Intr  101015  100926        -        0
   Intr  101223  101105        -        0
```

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```
                                                1462
      Intr  101620   101506          -          0
      Intr  101832   101736          -          0
      Intr  101991   101928          -          0
      Init  102199   102148          -          0

  >4584519        /6308

     len =      889    nex =         2

      Init   28246    28587          +          0
      Term   28683    29134          +          0

  >4584519        /28170

     len =     1464    nex =         6

      Term   34134    34075          -          0
      Intr   34343    34218          -          0
      Intr   34584    34431          -          0
      Intr   34767    34659          -          0
      Intr   34979    34916          -          0
      Init   35201    35079          -          0

  >4584519        /12980

     len =      612    nex =         1

      Sngl   38892    38281          -          0

  >4584531        /1259

     len =     1881    nex =         3

      Init    2041     2614          +          0
      Intr    3185     3256          +          0
      Term    3662     3921          +          0

  >4584531        /10016

     len =     1471    nex =         4

      Term   41067    40537          -          0
      Intr   41273    41234          -          0
      Intr   41510    41378          -          0
      Init   41872    41792          -          0

  >4584531        /42200

     len =      430    nex =         1

      Sngl   56875    56451          -          0

  >4584531        /38181

     len =     3698    nex =         4

      Term    4219     3881          -          0
      Intr    4842     4704          -          0
      Intr    5250     5029          -          0
      Init    7571     7406          -          0
```

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```
>4584841        /37069

    len =     1301   nex =        1

     Sngl    1552     252         -        0

>4584841        /16143

    len =     1050   nex =        2

     Term   75412   74936         -        0
     Init   75985   75751         -        0

>4584841        /159247

    len =      236   nex =        1

     Sngl   75985   75750         -        0

>4584841        /6145

    len =      910   nex =        2

     Term   75412   75090         -        0
     Init   75994   75751         -        0

>4584841        /123475

    len =      732   nex =        2

     Term   75412   75265         -        0
     Init   75996   75751         -        0

>4584841        /9874

    len =     1765   nex =        5

     Init   81507   81619         +        0
     Intr   81801   81961         +        0
     Intr   82057   82137         +        0
     Intr   82622   82745         +        0
     Term   82877   83265         +        0

>4585890        /120166

    len =      653   nex =        1

     Sngl   12573   11926         -        0

>4585890        /13800

    len =     2230   nex =        7

     Term   19173   18816         -        0
     Intr   19409   19311         -        0
     Intr   19822   19718         -        0
     Intr   20057   19911         -        0
     Intr   20227   20141         -        0
     Intr   20363   20307         -        0
     Init    21042   20494         -        0

>4585890        /23237

    len =     1936   nex =        8

     Term    21526   21333         -        0
     Intr    21725   21662         -        0
     Intr    21895   21830         -        0
     Intr    22074   22013         -        0
     Intr    22419   22332         -        0
     Intr    22565   22505         -        0
     Intr    22733   22650         -        0
     Init    23268   22886         -        0

>4585890        /101259

    len =     2530   nex =        9

     Init    27106   27159         +        0
     Intr    27272   27335         +        0
     Intr    27474   27530         +        0
     Intr    27682   27788         +        0
     Intr    27891   27929         +        0
     Intr    28113   28201         +        0
     Intr    28292   28370         +        0
     Intr    28459   28506         +        0
     Term    28596   28976         +        0

>4585890        /6787

    len =     1070   nex =        1

     Sngl    35922   34853         -        0

>4585890        /6066

    len =     1954   nex =        7

     Init    63417   63643         +        0
     Intr    63802   63857         +        0
     Intr    63959   64073         +        0
     Intr    64155   64265         +        0
     Intr    64360   64548         +        0
     Intr    64929   64985         +        0
     Term    65108   65370         +        0

>4585891        /33396

    len =     1691   nex =        3

     Term    13026   12969         -        0
     Intr    13198   13099         -        0
     Init    13882   13459         -        0

>4585891        /99687

    len =     1230   nex =        1

     Sngl    26998   27650         +        0
```

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```
>4585891        /38876

   len =     2154    nex =       8

    Term    57147    56769        -        0
    Intr    57348    57237        -        0
    Intr    57473    57439        -        0
    Intr    57784    57707        -        0
    Intr    58148    58008        -        0
    Intr    58290    58225        -        0
    Intr    58533    58389        -        0
    Init    58922    58608        -        0

>4585891        /108157

   len =      407    nex =       1

    Sngl     6299     5893        -        0

>4585891        /113049

   len =     1133    nex =       2

    Init    67122    67207        +        0
    Term    67928    68110        +        0

>4585891        /3006

   len =     2536    nex =      10

    Term    68750    68545        -        0
    Intr    68912    68844        -        0
    Intr    69081    69011        -        0
    Intr    69306    69228        -        0
    Intr    69556    69392        -        0
    Intr    69684    69640        -        0
    Intr    69849    69775        -        0
    Intr    70301    70125        -        0
    Intr    70649    70386        -        0
    Init    71080    70870        -        0

>4585891        /22546

   len =     1469    nex =       1

    Sngl    75226    73758        -        0

>4585891        /16254

   len =     1456    nex =       1

    Sngl    75226    73771        -        0

>4585891        /39762

   len =     1633    nex =       3

    Term     6306     5906        -        0
    Intr     6905     6416        -        0
```

09552156 .061502

```
                                   1466
   Init     7538    7127        -        0

>4585891        /38737

  len =     1846    nex =       3

   Term    87122   86839        -        0
   Intr    87408   87220        -        0
   Init    87854   87583        -        0

>4585896        /92372

  len =     1399    nex =       2

   Term     7865    7410        -        0
   Init     8808    8691        -        0

>4585896        /8

  len =     1584    nex =       2

   Term     7865    7424        -        0
   Init     8806    8691        -        0

>4585906        /21936

  len =      206    nex =       1

   Sngl    56292   56087        -        0

>4585918        /34035

  len =     1336    nex =       4

   Init     4050    4159        +        0
   Intr     4394    4474        +        0
   Intr     4559    4602        +        0
   Term     5076    5385        +        0

>4585952        /16644

  len =     1586    nex =       5

   Term    29418   29073        -        0
   Intr    29679   29554        -        0
   Intr    29904   29810        -        0
   Intr    30259   30174        -        0
   Init    30447   30407        -        0

>4585952        /25421

  len =     1909    nex =       6

   Term    29418   29073        -        0
   Intr    29679   29554        -        0
   Intr    29904   29810        -        0
   Intr    30259   30174        -        0
   Intr    30508   30407        -        0
   Init    30981   30796        -        0
```

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```
>4585952        /857

   len =      474    nex =        2

    Term   33620   33385          -          0
    Init   33853   33807          -          0

>4585952        /23092

   len =      568    nex =        1

    Sngl   39340   39907          +          0

>4585952        /205610

   len =      550    nex =        1

    Sngl   39349   39891          +          0

>4585952        /15789

   len =      430    nex =        1

    Sngl   39378   39802          +          0

>4585952        /11306

   len =     3130    nex =       12

    Term   45205   44960          -          0
    Intr   45443   45287          -          0
    Intr   45633   45542          -          0
    Intr   45776   45712          -          0
    Intr   45925   45860          -          0
    Intr   46136   46005          -          0
    Intr   46398   46225          -          0
    Intr   46643   46485          -          0
    Intr   46814   46727          -          0
    Intr   46979   46898          -          0
    Intr   47184   47067          -          0
    Init   47333   47268          -          0

>4585952        /20491

   len =     2610    nex =        7

    Term   60891   60337          -          0
    Intr   61479   61333          -          0
    Intr   61646   61593          -          0
    Intr   61921   61751          -          0
    Intr   62418   62209          -          0
    Intr   62609   62523          -          0
    Init   62935   62696          -          0

>4585952        /1496

   len =      610    nex =        1

    Sngl   91280   91884          +          0
```

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```
>4586024        /207026

    len =      970   nex =        2

     Term   24258   24031        -         0
     Init   24993   24849        -         0

>4586024        /39169

    len =     1138   nex =        2

     Init   25959   26001        +         0
     Term   26086   26319        +         0

>4586024        /24343

    len =      768   nex =        2

     Term   32828   32380        -         0
     Init   33147   32916        -         0

>4586024        /6387

    len =     2010   nex =        5

     Init   35746   35924        +         0
     Intr   36211   36327        +         0
     Intr   36439   36609        +         0
     Intr   36707   36919        +         0
     Term   37053   37444        +         0

>4586024        /6176

    len =     1690   nex =        5

     Init   35746   35924        +         0
     Intr   36211   36327        +         0
     Intr   36439   36609        +         0
     Intr   36707   36919        +         0
     Term   37053   37417        +         0

>4586024        /25528

    len =     1217   nex =        2

     Term   42248   41624        -         0
     Init   42832   42420        -         0

>4586024        /15198

    len =     1372   nex =        5

     Init    7326    7385        +         0
     Intr    7539    7640        +         0
     Intr    7863    7992        +         0
     Intr    8093    8205        +         0
     Term    8475    8697        +         0

>4586065        /41879
```

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```
len =     745   nex =        1

 Sngl   52682   51938         -        0

>4586098        /21839

len =    2814   nex =       10

 Term    8727    8320         -        0
 Intr    8910    8813         -        0
 Intr    9254    9144         -        0
 Intr    9412    9361         -        0
 Intr    9590    9514         -        0
 Intr    9773    9717         -        0
 Intr    9978    9878         -        0
 Intr   10163   10067         -        0
 Intr   10363   10251         -        0
 Init   11133   10862         -        0

>4586098        /2882

len =    1731   nex =        4

 Term   27031   26717         -        0
 Intr   27342   27125         -        0
 Intr   27658   27422         -        0
 Init   28447   27982         -        0

>4586098        /45

len =    1285   nex =        1

 Sngl   48639   49307         +        0

>4586098        /32461

len =    1772   nex =        5

 Term   61744   61488         -        0
 Intr   62495   62316         -        0
 Intr   62716   62607         -        0
 Intr   62865   62827         -        0
 Init   63259   62978         -        0

>4586098        /34781

len =    2678   nex =        5

 Init   65140   65679         +        0
 Intr   66213   66365         +        0
 Intr   66468   66604         +        0
 Intr   66694   66921         +        0
 Term   67078   67817         +        0

>4586098        /15574

len =    2030   nex =        5

 Init   72537   73258         +        0
 Intr   73723   73790         +        0
```

09555336.061700

```
        Intr   73883    74014         +         0
        Intr   74091    74252         +         0
        Term   74331    74555         +         0

>4586098        /35110

      len =     2352    nex =        5

        Init   87367    87850         +         0
        Intr   88069    88391         +         0
        Intr   88884    89031         +         0
        Intr   89142    89174         +         0
        Term   89266    89718         +         0

>4586241        /14095

      len =     1133    nex =        1

        Sngl   11254    12386         +         0

>4586241        /31570

      len =     2376    nex =        8

        Init   18202    18375         +         0
        Intr   18521    18586         +         0
        Intr   18671    18811         +         0
        Intr   18884    18961         +         0
        Intr   19370    19420         +         0
        Intr   19523    19557         +         0
        Intr   19641    19752         +         0
        Term   19852    20232         +         0

>4586241        /36486

      len =     1427    nex =        0

>4586241        /26799

      len =      474    nex =        1

        Sngl   24750    24277         -         0

>4586241        /12532

      len =     2237    nex =       10

        Term   47904    47677         -         0
        Intr   48038    47983         -         0
        Intr   48211    48118         -         0
        Intr   48426    48296         -         0
        Intr   48622    48519         -         0
        Intr   48849    48764         -         0
        Intr   49074    49005         -         0
        Intr   49267    49159         -         0
        Intr   49471    49364         -         0
        Init   49913    49842         -         0

>4586241        /34400
```

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```
      len =     1308    nex =         2

        Init   51838    52152          +         0
        Term   52518    53145          +         0

>4586241          /37484

      len =     4542    nex =         2

        Init   72812    73062          +         0
        Term   77290    77353          +         0

>4586241          /100613

      len =     4760    nex =         3

        Init   72812    73062          +         0
        Intr   77290    77355          +         0
        Term   77455    77571          +         0

>4586241          /516

      len =     5258    nex =         5

        Init   72812    73062          +         0
        Intr   77290    77355          +         0
        Intr   77455    77577          +         0
        Intr   77692    77784          +         0
        Term   77856    78069          +         0

>4586241          /41510

      len =      910    nex =         2

        Term   75339    74906          -         0
        Init   75807    75569          -         0

>4586241          /35907

      len =     1110    nex =         5

        Init   76894    77206          +         0
        Intr   77290    77355          +         0
        Intr   77455    77577          +         0
        Intr   77692    77784          +         0
        Term   77856    78003          +         0

>4586241          /17520

      len =      790    nex =         2

        Init    8059     8299          +         0
        Term    8495     8848          +         0

>4587582          /125961

      len =      490    nex =         1

        Sngl   13888    13411          -         0
```

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```
>4587582       /31554

   len =    1930   nex =       4

    Term   21991   21883        -        0
    Intr   22243   22097        -        0
    Intr   22707   22622        -        0
    Init   23280   23003        -        0

>4587582       /4595

   len =    1353   nex =       1

    Sngl   23593   24945        +        0

>4587582       /803

   len =    2112   nex =       2

    Init   26649   27735        +        0
    Term   28584   28760        +        0

>4587582       /16530

   len =    1096   nex =       2

    Term   29562   29488        -        0
    Init   30202   30172        -        0

>4587582       /103045

   len =    1270   nex =       2

    Term   29562   29325        -        0
    Init   30202   30172        -        0

>4587582       /10073

   len =    1665   nex =       2

    Term   29562   28975        -        0
    Init   30202   30172        -        0

>4587641       /19234

   len =     749   nex =       1

    Sngl  129286  128538        -        0

>4587641       /35921

   len =    2548   nex =       5

    Init  134987  135707        +        0
    Intr  135938  136040        +        0
    Intr  136602  136657        +        0
    Intr  136808  136972        +        0
    Term  137280  137534        +        0

>4587641       /29173
```

```
  len =     1616   nex =        4

   Init   26708   26786         +         0
   Intr   27042   27199         +         0
   Intr   27523   27614         +         0
   Term   27722   28051         +         0

>4587641        /20583

  len =     1234   nex =        4

   Init   26708   26786         +         0
   Intr   27042   27199         +         0
   Intr   27523   27614         +         0
   Term   27722   27934         +         0

>4587641        /3997

  len =     2393   nex =        4

   Init   31992   32060         +         0
   Intr   32147   32250         +         0
   Intr   32360   32790         +         0
   Term   32881   33710         +         0

>4587641        /9992

  len =     1188   nex =        4

   Init   57159   57189         +         0
   Intr   57295   57437         +         0
   Intr   57696   57818         +         0
   Term   57908   58346         +         0

>4587641        /41809

  len =     1062   nex =        3

   Init   60850   61072         +         0
   Intr   61195   61445         +         0
   Term   61534   61911         +         0

>4587641        /35151

  len =      649   nex =        2

   Term   81448   81337         -         0
   Init   81985   81802         -         0

>4587641        /12738

  len =      359   nex =        1

   Sngl   82158   81800         -         0

>4587641        /7074

  len =      690   nex =        1
```

```
    Sngl   82161   81802          -        0

>4587641        /108142

    len =     831   nex =        2

    Term   81448   81334          -        0
    Init   82164   81802          -        0

>4587677        /42117

    len =    1397   nex =        4

    Term    4964    4716          -        0
    Intr    5403    5201          -        0
    Intr    5650    5565          -        0
    Init    6112    5814          -        0

>4587986        /14922

    len =     561   nex =        1

    Sngl   19040   19589          +        0

>4587986        /6782

    len =     400   nex =        1

    Sngl   37458   37260          -        0

>4589409        /6760

    len =     715   nex =        2

    Init   15185   15608          +        0
    Term   15705   15899          +        0

>4589409        /15004

    len =     730   nex =        1

    Sngl   22595   21875          -        0

>4589409        /29063

    len =    1952   nex =        4

    Term   23209   22949          -        0
    Intr   23499   23305          -        0
    Intr   23954   23842          -        0
    Init   24900   24844          -        0

>4589409        /16621

    len =    2096   nex =        5

    Init   25892   26032          +        0
    Intr   26337   26445          +        0
    Intr   26520   26581          +        0
    Intr   26672   26775          +        0
```

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```
     Term   26873   27227        +        0

  >4589409        /28003

     len =    1663    nex =        2

     Init   33218   33581        +        0
     Term   34356   34880        +        0

  >4589409        /32842

     len =    1305    nex =        1

     Sngl   36996   38300        +        0

  >4589410        /41421

     len =    1995    nex =        4

     Init   13570   13637        +        0
     Intr   13729   14122        +        0
     Intr   14271   14884        +        0
     Term   14990   15286        +        0

  >4589410        /6022

     len =    1463    nex =        3

     Init   13320   13637        +        0
     Intr   13729   14122        +        0
     Term   14271   14782        +        0

  >4589410        /31579

     len =    3250    nex =       10

     Init   25051   25297        +        0
     Intr   25751   25844        +        0
     Intr   25940   26056        +        0
     Intr   26147   26219        +        0
     Intr   26327   26445        +        0
     Intr   26548   26669        +        0
     Intr   26906   27082        +        0
     Intr   27194   27338        +        0
     Intr   27491   27669        +        0
     Term   27843   28293        +        0

  >4589410        /32783

     len =     716    nex =        3

     Init   32322   32516        +        0
     Intr   32607   32735        +        0
     Term   32829   32876        +        0

  >4589410        /22152

     len =    1661    nex =        5

     Term   40659   40201        -        0
```

```
        Intr    40967   40894         -       0
        Intr    41125   41044         -       0
        Intr    41359   41262         -       0
        Init    41861   41719         -       0

    >4589410       /38797

      len =     1392    nex =       1

        Sngl    49398   50789         +       0

    >4589410       /157709

      len =      623    nex =       1

        Sngl    53543   54165         +       0

    >4589410       /21863

      len =     1341    nex =       1

        Sngl    63197   61857         -       0

    >4589410       /41153

      len =     1351    nex =       2

        Init    67494   68408         +       0
        Term    68630   68844         +       0

    >4589411       /2612

      len =      795    nex =       1

        Sngl    11554   11467         -       0

    >4589411       /12760

      len =     1958    nex =       6

        Term    11151   10758         -       0
        Intr    11356   11253         -       0
        Intr    11566   11467         -       0
        Intr    12211   12152         -       0
        Intr    12387   12316         -       0
        Init    12562   12491         -       0

    >4589411       /7631

      len =     2679    nex =       9

        Term    11151   10921         -       0
        Intr    11356   11253         -       0
        Intr    11566   11467         -       0
        Intr    12211   12152         -       0
        Intr    12387   12316         -       0
        Intr    12562   12491         -       0
        Intr    12737   12645         -       0
        Intr    12928   12861         -       0
        Init    13599   13285         -       0
```

```
>4589412        /144945

   len =     1238    nex =        4

    Init   13026    13085          +         0
    Intr   13230    13310          +         0
    Intr   13425    13664          +         0
    Term   13744    14126          +         0

>4589412        /25062

   len =     2039    nex =        3

    Term   14762    14637          -         0
    Intr   15769    15710          -         0
    Init   16311    15854          -         0

>4589412        /22430

   len =     4909    nex =       15

    Init   17362    17458          +         0
    Intr   17622    17795          +         0
    Intr   17885    17936          +         0
    Intr   18026    18133          +         0
    Intr   18262    18346          +         0
    Intr   18636    18690          +         0
    Intr   18783    19226          +         0
    Intr   19904    19963          +         0
    Intr   20194    20292          +         0
    Intr   20386    20496          +         0
    Intr   20651    20722          +         0
    Intr   20815    20931          +         0
    Intr   21132    21281          +         0
    Intr   21415    21515          +         0
    Term   21862    22270          +         0

>4589412        /92349

   len =     1260    nex =        4

    Term    1333      917          -         0
    Intr    1647     1400          -         0
    Intr    2010     1852          -         0
    Init    2176     2133          -         0

>4589412        /6550

   len =      940    nex =        3

    Term   35193    34956          -         0
    Intr   35603    35471          -         0
    Init   35895    35698          -         0

>4589412        /91769

   len =     1450    nex =        5

    Term   35193    34965          -         0
```

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```
    Intr   35603   35471          -        0
    Intr   35977   35698          -        0
    Intr   36224   36176          -        0
    Init   36412   36339          -        0

>4589412        /37702

  len =     2953   nex =        8

    Term   38862   38499          -        0
    Intr   39017   38958          -        0
    Intr   39247   39138          -        0
    Intr   39446   39339          -        0
    Intr   39725   39558          -        0
    Intr   39942   39819          -        0
    Intr   40327   40270          -        0
    Init   41451   41110          -        0

>4589412        /19542

  len =      859   nex =        3

    Init   63381   63483          +        0
    Intr   63632   63707          +        0
    Term   63802   64239          +        0

>4589412        /26264

  len =      600   nex =        1

    Sngl   70812   70213          -        0

>4589414        /92314

  len =      790   nex =        1

    Sngl   27496   26709          -        0

>4589414        /21068

  len =      860   nex =        1

    Sngl   54424   55283          +        0

>4589414        /41648

  len =     2894   nex =       10

    Init   80194   80450          +        0
    Intr   80547   80661          +        0
    Intr   80904   80984          +        0
    Intr   81081   81169          +        0
    Intr   81271   81362          +        0
    Intr   81484   81565          +        0
    Intr   81673   81775          +        0
    Intr   81860   81931          +        0
    Intr   82124   82209          +        0
    Term   82304   83087          +        0

>4589415        /4507
```

```
    len =      855    nex =        2

     Init        1       35         +         0
     Term      299      855         +         0

>4589415        /117537

    len =     2514    nex =        7

     Term    12799    12501         -         0
     Intr    12999    12887         -         0
     Intr    13168    13088         -         0
     Intr    13342    13290         -         0
     Intr    13544    13436         -         0
     Intr    13699    13640         -         0
     Init    14105    14023         -         0

>4589415        /159318

    len =     1296    nex =        3

     Term    17311    16832         -         0
     Intr    17535    17398         -         0
     Init    18127    17694         -         0

>4589415        /36400

    len =     1837    nex =        6

     Init    18863    19024         +         0
     Intr    19422    19565         +         0
     Intr    19664    19755         +         0
     Intr    19856    20042         +         0
     Intr    20135    20290         +         0
     Term    20375    20699         +         0

>4589418        /12825

    len =     1367    nex =        4

     Term       91        1         -         0
     Intr      499      185         -         0
     Intr     1228      970         -         0
     Init     1367     1302         -         0

>4589418        /13931

    len =     2006    nex =        9

     Term    12022    11700         -         0
     Intr    12272    12099         -         0
     Intr    12470    12369         -         0
     Intr    12618    12554         -         0
     Intr    12784    12701         -         0
     Intr    12976    12904         -         0
     Intr    13171    13070         -         0
     Intr    13490    13371         -         0
     Init    13705    13592         -         0
```

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```
>4589418        /8249

   len =     3294    nex =       13

    Init   21324    21424          +         0
    Intr   21529    21612          +         0
    Intr   21826    21872          +         0
    Intr   22163    22237          +         0
    Intr   22318    22395          +         0
    Intr   22608    22670          +         0
    Intr   22801    22871          +         0
    Intr   22975    23074          +         0
    Intr   23213    23317          +         0
    Intr   23570    23632          +         0
    Intr   23717    23839          +         0
    Intr   23953    24105          +         0
    Term   24189    24497          +         0

>4589419        /24081

   len =      550    nex =        1

    Sngl   32416    32957          +         0

>4589419        /106001

   len =      790    nex =        3

    Init   32416    32802          +         0
    Intr   32996    33100          +         0
    Term   33137    33197          +         0

>4589419        /24665

   len =      730    nex =        1

    Sngl   35180    34460          -         0

>4589419        /21922

   len =     2840    nex =        6

    Term   35177    34454          -         0
    Intr   35520    35348          -         0
    Intr   35757    35622          -         0
    Intr   36338    35874          -         0
    Intr   36722    36412          -         0
    Init   37293    36801          -         0

>4589419        /10037

   len =      679    nex =        2

    Term   42723    42430          -         0
    Init   43108    42926          -         0

>4589421        /28511

   len =      984    nex =        5
```

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```
      Term    9429     9171          -        0
      Intr    9603     9520          -        0
      Intr    9780     9688          -        0
      Intr    9922     9850          -        0
      Init   10144    10006          -        0

   >4589421       /32718

     len =     1303    nex =        5

      Term   25886    25513          -        0
      Intr   26219    26136          -        0
      Intr   26411    26319          -        0
      Intr   26577    26505          -        0
      Init   26815    26651          -        0

   >4589421       /5240

     len =      641    nex =        1

      Sngl   61499    62139          +        0

   >4589421       /20850

     len =     2056    nex =        3

      Term   61249    60746          -        0
      Intr   61952    61661          -        0
      Init   62801    62305          -        0

   >4589421       /11329

     len =      442    nex =        1

      Sngl   80041    79600          -        0

   >4589423       /33833

     len =      850    nex =        2

      Term   14120    13771          -        0
      Init   14612    14518          -        0

   >4589423       /36859

     len =      854    nex =        2

      Term   14120    13819          -        0
      Init   14672    14518          -        0

   >4589425       /113229

     len =      511    nex =        1

      Sngl   33439    32996          -        0

   >4589427       /1076

     len =      498    nex =        1
```

```
                                        1482
     Sngl    25047    24550         -      0

  >4589427        /17208

     len =     1482    nex =       4

     Term    25597    25201         -      0
     Intr    25959    25691         -      0
     Intr    26181    26067         -      0
     Init    26540    26433         -      0

  >4589428        /33021

     len =     3085    nex =      11

     Init    11298    11398         +      0
     Intr    12000    12227         +      0
     Intr    12319    12366         +      0
     Intr    12551    12627         +      0
     Intr    12718    12840         +      0
     Intr    13160    13264         +      0
     Intr    13353    13425         +      0
     Intr    13510    13581         +      0
     Intr    13702    13894         +      0
     Intr    13978    14107         +      0
     Term    14194    14382         +      0

  >4589428        /39126

     len =     2513    nex =      10

     Init    12000    12227         +      0
     Intr    12319    12366         +      0
     Intr    12551    12627         +      0
     Intr    12718    12840         +      0
     Intr    13160    13264         +      0
     Intr    13353    13425         +      0
     Intr    13510    13581         +      0
     Intr    13702    13894         +      0
     Intr    13978    14107         +      0
     Term    14194    14381         +      0

  >4589428        /38630

     len =     1819    nex =       4

     Init    27537    27623         +      0
     Intr    28018    28109         +      0
     Intr    28242    28316         +      0
     Term    28403    29075         +      0

  >4589428        /21695

     len =     2374    nex =       7

     Init     2805     3043         +      0
     Intr     3162     3288         +      0
     Intr     3751     3868         +      0
     Intr     3992     4128         +      0
     Intr     4215     4316         +      0
```

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```
     Intr    4412    4504        +       0
     Term    4600    5178        +       0

>4589428       /1326

   len =    1476   nex =       3

     Init   37323   37772        +       0
     Intr   38054   38269        +       0
     Term   38350   38798        +       0

>4589428       /28772

   len =    1258   nex =       4

     Term   39781   39356        -       0
     Intr   39944   39891        -       0
     Intr   40129   40040        -       0
     Init   40613   40330        -       0

>4589428       /22839

   len =     496   nex =       1

     Sngl    6020    5525        -       0

>4589430       /37580

   len =     134   nex =       1

     Sngl   20755   20888        +       0

>4589430       /35379

   len =    1546   nex =       5

     Init   36633   36700        +       0
     Intr   36804   37042        +       0
     Intr   37150   37290        +       0
     Intr   37379   37813        +       0
     Term   37902   38175        +       0

>4589430       /26766

   len =     430   nex =       1

     Sngl   37902   38175        +       0

>4589430       /4357

   len =    2437   nex =       5

     Init   57993   58176        +       0
     Intr   58292   58405        +       0
     Intr   59163   59390        +       0
     Intr   59478   59917        +       0
     Term   60008   60429        +       0

>4589430       /37065
```

```
     len =     1885    nex =        4

       Init   60646    60889         +         0
       Intr   61155    61335         +         0
       Intr   61583    61724         +         0
       Term   61827    61896         +         0

>4589430        /110428

     len =      535    nex =        2

       Term   72697    72339         -         0
       Init   72873    72781         -         0

>4589432        /3332

     len =      771    nex =        2

       Term   15502    15080         -         0
       Init   15850    15746         -         0

>4589432        /123678

     len =     1078    nex =        2

       Init   21735    22234         +         0
       Term   22314    22812         +         0

>4589432        /40179

     len =     2617    nex =        5

       Term   43904    43354         -         0
       Intr   44457    44374         -         0
       Intr   44853    44548         -         0
       Intr   45547    45471         -         0
       Init   45970    45638         -         0

>4589433        /12315

     len =      194    nex =        1

       Sngl   10614    10421         -         0

>4589434        /28462

     len =     1771    nex =        2

       Init   10665    10924         +         0
       Term   11022    11140         +         0

>4589434        /104779

     len =     1639    nex =        2

       Init   10667    10924         +         0
       Term   11022    11140         +         0

>4589434        /108581
```

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```
      len =     1690    nex =        3

       Init   10667   10924          +        0
       Intr   11022   11140          +        0
       Term   11602   11648          +        0

   >4589434        /33570

      len =     1638    nex =        2

       Init   10684   10924          +        0
       Term   11022   11140          +        0

   >4589434        /9336

      len =      599    nex =        1

       Sngl    1164     566          -        0

   >4589434        /39717

      len =     1792    nex =        5

       Term   31362   31004          -        0
       Intr   31686   31448          -        0
       Intr   32066   31860          -        0
       Intr   32345   32156          -        0
       Init   32795   32494          -        0

   >4589434        /6827

      len =     2290    nex =        1

       Sngl   37865   37353          -        0

   >4589434        /7571

      len =     2455    nex =        8

       Term   43285   43064          -        0
       Intr   43481   43379          -        0
       Intr   43949   43881          -        0
       Intr   44126   44067          -        0
       Intr   44318   44225          -        0
       Intr   44720   44603          -        0
       Intr   44970   44809          -        0
       Init   45518   45143          -        0

   >4589434        /31680

      len =      550    nex =        1

       Sngl   46938   46393          -        0

   >4589434        /12344

      len =     2121    nex =        4

       Term   52868   52559          -        0
       Intr   54223   54128          -        0
      Intr    54396   54310          -         0
      Init    54679   54472          -         0

   >4589434        /15457

     len =      626   nex =        1

      Sngl    61850   61225          -         0

   >4589434        /1876

     len =     2436   nex =        4

      Term    62504   62245          -         0
      Intr    63661   62602          -         0
      Intr    64223   64000          -         0
      Init    64396   64311          -         0

   >4589434        /159403

     len =      850   nex =        2

      Term    67516   66998          -         0
      Init    67838   67591          -         0

   >4589434        /15623

     len =     1390   nex =        2

      Term    76523   76081          -         0
      Init    77465   76628          -         0

   >4589435        /24826

     len =      610   nex =        1

      Sngl    21600   20996          -         0

   >4589435        /6424

     len =     2470   nex =       10

      Init    29316   29493          +         0
      Intr    29595   29735          +         0
      Intr    29824   29872          +         0
      Intr    30020   30037          +         0
      Intr    30228   30343          +         0
      Intr    30437   30550          +         0
      Intr    30867   30955          +         0
      Intr    31183   31260          +         0
      Intr    31353   31460          +         0
      Term    31549   31779          +         0

   >4589435        /10035

     len =     1247   nex =        4

      Init    37688   37775          +         0
      Intr    37871   38014          +         0
      Intr    38315   38407          +         0
```

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```
    Term   38519   38934         +        0

>4589435       /23983

   len =     730   nex =       1

    Sngl   42012   42734         +        0

>4589435       /22609

   len =     459   nex =       1

    Sngl   69525   69067         -        0

>4589435       /93294

   len =    1273   nex =       5

    Term   70107   69919         -        0
    Intr   70459   70342         -        0
    Intr   70608   70552         -        0
    Intr   70794   70727         -        0
    Init   71191   70923         -        0

>4589436       /40348

   len =    1614   nex =       4

    Term   16780   16494         -        0
    Intr   17196   17147         -        0
    Intr   17517   17283         -        0
    Init   18107   17946         -        0

>4589436       /6991

   len =    3085   nex =       6

    Init   29559   29939         +        0
    Intr   29982   30049         +        0
    Intr   30155   30401         +        0
    Intr   30498   30792         +        0
    Intr   30872   31246         +        0
    Term   31592   32643         +        0

>4589436       /2373

   len =     769   nex =       1

    Sngl   31994   32762         +        0

>4589436       /34946

   len =    2208   nex =       9

    Init   38601   38660         +        0
    Intr   38850   38896         +        0
    Intr   39015   39108         +        0
    Intr   39199   39346         +        0
    Intr   39507   39719         +        0
    Intr   39820   39954         +        0
```

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```
        Intr   40047   40181         +        0
        Intr   40269   40484         +        0
        Term   40687   40808         +        0

    >4589437       /42922

       len =    1778   nex =       2

        Term   10942   10397         -        0
        Init   11556   11034         -        0

    >4589437       /92639

       len =     610   nex =       1

        Sngl   55996   55836         -        0

    >4589437       /20558

       len =    2456   nex =       5

        Term   55692   55343         -        0
        Intr   55970   55836         -        0
        Intr   56286   56230         -        0
        Intr   56657   56523         -        0
        Init   57335   57057         -        0

    >4589437       /108568

       len =    3071   nex =       7

        Init   59166   59592         +        0
        Intr   59799   60132         +        0
        Intr   60223   60263         +        0
        Intr   60986   61027         +        0
        Intr   61230   61351         +        0
        Intr   61440   61548         +        0
        Term   62046   62236         +        0

    >4589437       /34275

       len =    2455   nex =       0

    >4589437       /14275

       len =     840   nex =       1

        Sngl    8228    7573         -        0

    >4589437       /41112

       len =    1440   nex =       7

        Init    8889    9041         +        0
        Intr    9162    9359         +        0
        Intr    9462    9551         +        0
        Intr    9629    9718         +        0
        Intr    9803    9883         +        0
        Intr   10024   10069         +        0
        Term   10176   10328         +        0
```

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```
>4589438       /25545

    len =     1215   nex =       2

      Term   24727   24486         -         0
      Init   25352   25278         -         0

>4589438       /30064

    len =     1570   nex =       5

      Init   29309   29481         +         0
      Intr   29563   29748         +         0
      Intr   29831   29993         +         0
      Intr   30080   30266         +         0
      Term   30349   30622         +         0

>4589438       /21843

    len =      989   nex =       1

      Sngl   46380   47368         +         0

>4589438       /22434

    len =     1518   nex =       4

      Init    5568    5639         +         0
      Intr    5947    6024         +         0
      Intr    6128    6406         +         0
      Term    6506    7066         +         0

>4589439       /10004

    len =      463   nex =       1

      Sngl   40998   41460         +         0

>4589439       /26026

    len =      670   nex =       1

      Sngl   46310   45649         -         0

>4589439       /41488

    len =      970   nex =       2

      Init   47658   48053         +         0
      Term   48141   48323         +         0

>4589439       /23276

    len =     1822   nex =       6

      Term   63167   62825         -         0
      Intr   63423   63256         -         0
      Intr   63691   63515         -         0
      Intr   63954   63873         -         0
    Intr   64223   64046         -       0
    Init   64646   64414         -       0

>4589439       /25458

   len =    1950   nex =       5

    Term   65270   64949         -       0
    Intr   65611   65475         -       0
    Intr   65803   65700         -       0
    Intr   66294   66199         -       0
    Init   66470   66385         -       0

>4589439       /9482

   len =    1129   nex =       1

    Sngl   67879   69007         +       0

>4589439       /42187

   len =    1190   nex =       1

    Sngl   67901   69090         +       0

>4589440       /30989

   len =    1881   nex =       5

    Init    1357    1831         +       0
    Intr    1938    2061         +       0
    Intr    2142    2278         +       0
    Intr    2717    2800         +       0
    Term    2908    3237         +       0

>4589440       /114071

   len =     537   nex =       0

>4589443       /35696

   len =    2052   nex =       8

    Init   10117   10334         +       0
    Intr   10710   10812         +       0
    Intr   10911   11018         +       0
    Intr   11125   11234         +       0
    Intr   11323   11430         +       0
    Intr   11525   11570         +       0
    Intr   11688   11819         +       0
    Term   11918   12161         +       0

>4589443       /40267

   len =    1345   nex =       5

    Term   32380   32218         -       0
    Intr   32541   32485         -       0
    Intr   32744   32640         -       0
    Intr   32964   32857         -       0
```

```
     Init   33319   33125         -        0

>4589443       /144066

   len =    1510   nex =       3

     Term   37967   37540         -        0
     Intr   38213   38133         -        0
     Init   39044   38777         -        0

>4589444       /40174

   len =    2138   nex =       7

     Init    5581    5696         +        0
     Intr    6020    6178         +        0
     Intr    6278    6453         +        0
     Intr    6565    6842         +        0
     Intr    6928    7063         +        0
     Intr    7152    7263         +        0
     Term    7457    7718         +        0

>4589444       /120707

   len =    1653   nex =       5

     Init   68700   68826         +        0
     Intr   68906   69190         +        0
     Intr   69489   69569         +        0
     Intr   69673   69767         +        0
     Term   69988   70352         +        0

>4589444       /10687

   len =     582   nex =       2

     Init    7159    7263         +        0
     Term    7457    7740         +        0

>4589444       /13338

   len =    1435   nex =       2

     Term   71110   70333         -        0
     Init   71767   71187         -        0

>4589444       /12533

   len =    1612   nex =       3

     Term   72674   72238         -        0
     Intr   73073   72743         -        0
     Init   73849   73302         -        0

>4589444       /327

   len =    1816   nex =       5

     Init   74986   75176         +        0
     Intr   75871   75973         +        0
```

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```
      Intr   76059   76148        +       0
      Intr   76241   76339        +       0
      Term   76490   76801        +       0

>4589445        /17344

    len =    1030   nex =       2

      Init       1     139        +       0
      Term     623    1027        +       0

>4589445        /38265

    len =    2260   nex =       9

      Init   19710   19895        +       0
      Intr   20156   20228        +       0
      Intr   20310   20392        +       0
      Intr   20491   20593        +       0
      Intr   20692   20848        +       0
      Intr   20926   21054        +       0
      Intr   21150   21236        +       0
      Intr   21401   21445        +       0
      Term   21645   21969        +       0

>4589445        /40038

    len =    1410   nex =       1

      Sngl   23376   22781        -       0

>4589445        /40717

    len =    1171   nex =       6

      Term   59227   59185        -       0
      Intr   59432   59317        -       0
      Intr   59660   59559        -       0
      Intr   59846   59745        -       0
      Intr   60036   59926        -       0
      Init   60355   60179        -       0

>4589445        /6617

    len =    1852   nex =       4

      Init   79257   79346        +       0
      Intr   79428   79703        +       0
      Intr   80505   80663        +       0
      Term   80741   81108        +       0

>4589446        /20765

    len =    2682   nex =       8

      Term   14307   14077        -       0
      Intr   14445   14401        -       0
      Intr   14633   14514        -       0
      Intr   14989   14901        -       0
      Intr   15212   15134        -       0
```

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```
      Intr   15616   15454        -        0
      Intr   15937   15858        -        0
      Init   16118   16011        -        0

>4589446        /38867

   len =     1907   nex =       4

      Init   17211   17769        +        0
      Intr   18004   18141        +        0
      Intr   18241   18490        +        0
      Term   18625   19117        +        0

>4589450        /124077

   len =     1690   nex =       6

      Init    6092    6223        +        0
      Intr    6597    6755        +        0
      Intr    7007    7256        +        0
      Intr    7347    7436        +        0
      Intr    7529    7617        +        0
      Term    7744    7770        +        0

>4589950        /6704

   len =     2072   nex =       5

      Init   15942   16097        +        0
      Intr   16230   16315        +        0
      Intr   16673   16798        +        0
      Intr   16932   17059        +        0
      Term   17165   17489        +        0

>4589950        /152803

   len =      611   nex =       1

      Sngl   23934   24544        +        0

>4589950        /38094

   len =      326   nex =       1

      Sngl   24221   24542        +        0

>4589950        /20770

   len =     1643   nex =       4

      Init   27913   28123        +        0
      Intr   28278   28494        +        0
      Intr   28594   28778        +        0
      Term   29119   29555        +        0

>4589950        /17664

   len =     2673   nex =       4

      Init   30408   30899        +        0
```

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```
         Intr   31702   32034          +        0
         Intr   32610   32726          +        0
         Term   32854   33080          +        0

>4589950        /21841

       len =     1630   nex =        5

         Init   43014   43406          +        0
         Intr   43536   43676          +        0
         Intr   43768   43908          +        0
         Intr   44011   44117          +        0
         Term   44298   44634          +        0

>4589950        /11283

       len =     1270   nex =        2

         Term    3662    3371          -        0
         Init    4626    4390          -        0

>4589950        /100317

       len =     1255   nex =        2

         Term    3662    3416          -        0
         Init    4670    4390          -        0

>4589950        /41633

       len =      569   nex =        1

         Sngl   50817   51385          +        0

>4589969        /120267

       len =      610   nex =        2

         Init   40423   40571          +        0
         Term   40797   41025          +        0

>4646215        /34289

       len =      776   nex =        3

         Init   19221   19325          +        0
         Intr   19412   19533          +        0
         Term   19667   19993          +        0

>4646215        /9248

       len =      867   nex =        1

         Sngl   21032   20166          -        0

>4646229        /103197

       len =      751   nex =        2

         Init    3186    3298          +        0
```

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```
    Term    3509    3709         +        0

>4646229       /19631

   len =      691   nex =       1

    Sngl   56338   57028         +        0

>4646229       /2267

   len =     2371   nex =       7

    Init   59713   59825         +        0
    Intr   59944   59982         +        0
    Intr   60076   60125         +        0
    Intr   60872   60983         +        0
    Intr   61359   61406         +        0
    Intr   61583   61713         +        0
    Term   61829   61997         +        0

>4646229       /6261

   len =      408   nex =       1

    Sngl   64632   65028         +        0

>4662609       /24161

   len =     1541   nex =       5

    Init  110615  110883         +        0
    Intr  111111  111219         +        0
    Intr  111307  111437         +        0
    Intr  111538  111612         +        0
    Term  111789  112155         +        0

>4662609       /796

   len =      618   nex =       2

    Init  110728  110883         +        0
    Term  111111  111219         +        0

>4662609       /18583

   len =     1090   nex =       6

    Init   11288   11484         +        0
    Intr   11708   11848         +        0
    Intr   11922   12002         +        0
    Intr   12074   12198         +        0
    Intr   12253   12295         +        0
    Term   12327   12377         +        0

>4662609       /32558

   len =     2255   nex =       5

    Init  119155  119438         +        0
    Intr  119536  119598         +        0
```

```
     Intr  120052  120472        +        0
     Intr  120567  120718        +        0
     Term  120832  121409        +        0

>4662609        /103288

   len =     1570    nex =       6

     Init    42796    42919       +        0
     Intr    43094    43245       +        0
     Intr    43358    43467       +        0
     Intr    43556    43641       +        0
     Intr    43725    43829       +        0
     Term    43910    44068       +        0

>4662609        /7803

   len =      379    nex =       2

     Term    58481    58268       -        0
     Init    58640    58572       -        0

>4662609        /34358

   len =      873    nex =       1

     Sngl    75547    76419       +        0

>4662628        /9376

   len =     1657    nex =       4

     Init    31339    31677       +        0
     Intr    31931    32188       +        0
     Intr    32461    32664       +        0
     Term    32749    32995       +        0

>4662628        /154050

   len =     1099    nex =       2

     Term    36454    36398       -        0
     Init    37496    37156       -        0

>4662628        /20900

   len =     1390    nex =       1

     Sngl     7708     9057       +        0

>4662637        /20182

   len =     2859    nex =       7

     Term    28149    27892       -        0
     Intr    28599    28528       -        0
     Intr    28829    28763       -        0
     Intr    29070    28932       -        0
     Intr    29862    29688       -        0
     Intr    30330    30177       -        0
```

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```
    Init   30750   30535         -        0

>4662640        /103735

   len =     670   nex =        1

    Sngl    1875    1670         -        0

>4662640        /13193

   len =     741   nex =        1

    Sngl   24672   23932         -        0

>4662640        /108284

   len =    1342   nex =        2

    Term    1875    1599         -        0
    Init    2933    2230         -        0

>4662640        /32647

   len =    1474   nex =        2

    Term    1875    1671         -        0
    Init    3144    2230         -        0

>4662640        /111177

   len =     370   nex =        1

    Sngl    3147    2785         -        0

>4662640        /32660

   len =    2432   nex =        3

    Term    7037    6524         -        0
    Intr    7476    7292         -        0
    Init    8955    8479         -        0

>4678196        /1940

   len =     830   nex =        4

    Term   30655   30442         -        0
    Intr   30869   30753         -        0
    Intr   31063   30949         -        0
    Init   31271   31146         -        0

>4678196        /30469

   len =     858   nex =        4

    Term   30655   30458         -        0
    Intr   30869   30753         -        0
    Intr   31063   30949         -        0
    Init   31315   31146         -        0
```

```
>4678196        /18195

   len =      984    nex =        4

    Term   30655   30384          -        0
    Intr   30869   30753          -        0
    Intr   31063   30949          -        0
    Init   31367   31146          -        0

>4678196        /994

   len =     1046    nex =        4

    Term    7202    6888          -        0
    Intr    7473    7304          -        0
    Intr    7664    7566          -        0
    Init    7933    7778          -        0

>4678219        /21113

   len =      414    nex =        1

    Sngl   16445   16858          +        0

>4678219        /124189

   len =     1617    nex =        6

    Init   17224   17328          +        0
    Intr   17546   17641          +        0
    Intr   17745   17806          +        0
    Intr   18172   18305          +        0
    Intr   18426   18510          +        0
    Term   18608   18840          +        0

>4678219        /10500

   len =      970    nex =        3

    Init   18935   19234          +        0
    Intr   19376   19429          +        0
    Term   19531   19898          +        0

>4678219        /41471

   len =     1498    nex =        6

    Init   20212   20321          +        0
    Intr   20661   20780          +        0
    Intr   20859   20904          +        0
    Intr   21091   21176          +        0
    Intr   21267   21396          +        0
    Term   21486   21709          +        0

>4678219        /1918

   len =     1090    nex =        5

    Init   22504   22649          +        0
    Intr   22728   22814          +        0
```

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```
    Intr   22918   23020        +        0
    Intr   23256   23322        +        0
    Term   23414   23593        +        0

>4678258       /38927

   len =    2446   nex =       8

    Term   35527   35138        -        0
    Intr   35817   35649        -        0
    Intr   36025   35917        -        0
    Intr   36381   36162        -        0
    Intr   36617   36472        -        0
    Intr   36786   36696        -        0
    Intr   37244   37130        -        0
    Init   37583   37343        -        0

>4678258       /2632

   len =    2950   nex =      10

    Term   38519   37849        -        0
    Intr   38936   38848        -        0
    Intr   39250   39065        -        0
    Intr   39398   39336        -        0
    Intr   39714   39532        -        0
    Intr   39870   39822        -        0
    Intr   40128   39990        -        0
    Intr   40328   40247        -        0
    Intr   40471   40416        -        0
    Init   40791   40667        -        0

>4678258       /30342

   len =     533   nex =       1

    Sngl   88640   89172        +        0

>4678266       /19033

   len =     577   nex =       1

    Sngl    1664    2240        +        0

>4678266       /22956

   len =     642   nex =       1

    Sngl    4832    5473        +        0

>4678266       /13259

   len =    2274   nex =       4

    Init   73401   73742        +        0
    Intr   73945   74637        +        0
    Intr   74813   75124        +        0
    Term   75360   75674        +        0

>4678266       /332
```

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```
      len =     1090    nex =        1

       Sngl    77266    76186          -         0

>4678266        /27474

      len =     2792    nex =       10

       Term    90126    89784          -         0
       Intr    90502    90362          -         0
       Intr    90835    90594          -         0
       Intr    91028    90903          -         0
       Intr    91211    91118          -         0
       Intr    91432    91331          -         0
       Intr    91850    91778          -         0
       Intr    91990    91933          -         0
       Intr    92372    92188          -         0
       Init    92566    92480          -         0

>4678291        /19152

      len =     1917    nex =        7

       Init    17191    17291          +         0
       Intr    17368    17833          +         0
       Intr    17968    18085          +         0
       Intr    18232    18341          +         0
       Intr    18427    18512          +         0
       Intr    18635    18780          +         0
       Term    18898    19107          +         0

>4678291        /30751

      len =     1344    nex =        7

       Init    22561    22613          +         0
       Intr    22732    22806          +         0
       Intr    22896    22964          +         0
       Intr    23038    23118          +         0
       Intr    23250    23345          +         0
       Intr    23435    23491          +         0
       Term    23601    23896          +         0

>4678291        /33606

      len =      446    nex =        1

       Sngl    23601    23896          +         0

>4678291        /5493

      len =     1270    nex =        2

       Init    27193    27656          +         0
       Term    27889    28459          +         0

>4678291        /37225

      len =     2003    nex =        3
```

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```
    Init    42970   43714         +         0
    Intr    43777   43917         +         0
    Term    44045   44522         +         0

>4678291        /21763

   len =     933    nex =        3

    Init    46242   46546         +         0
    Intr    46860   46962         +         0
    Term    47050   47174         +         0

>4678291        /25149

   len =    2552    nex =        8

    Term    66537   66278         -         0
    Intr    66874   66788         -         0
    Intr    67068   66979         -         0
    Intr    67487   67382         -         0
    Intr    67678   67584         -         0
    Intr    67904   67821         -         0
    Intr    68418   68343         -         0
    Init    68829   68689         -         0

>4678291        /15212

   len =    2530    nex =        7

    Term    66537   66335         -         0
    Intr    66874   66788         -         0
    Intr    67068   66979         -         0
    Intr    67487   67382         -         0
    Intr    67678   67584         -         0
    Intr    67904   67821         -         0
    Init    68422   68343         -         0

>4678315        /34579

   len =    1990    nex =        5

    Init    13656   13884         +         0
    Intr    13988   14067         +         0
    Intr    14162   14239         +         0
    Intr    14367   14437         +         0
    Term    15188   15636         +         0

>4678315        /787

   len =     324    nex =        1

    Sngl    18778   19101         +         0

>4678315        /147241

   len =     341    nex =        1

    Sngl     2959    3299         +         0
```

```
>4678315       /34551

   len =    1545   nex =       7

     Term   41502   41430        -        0
     Intr   41669   41604        -        0
     Intr   41835   41771        -        0
     Intr   41994   41931        -        0
     Intr   42197   42088        -        0
     Intr   42483   42396        -        0
     Init   42974   42809        -        0

>4678315       /9581

   len =    1336   nex =       2

     Init   46422   46595        +        0
     Term   47332   47757        +        0

>4678340       /12236

   len =    1954   nex =       5

     Term   22952   22722        -        0
     Intr   23125   23045        -        0
     Intr   23705   23209        -        0
     Intr   24098   23940        -        0
     Init   24675   24184        -        0

>4678340       /26537

   len =    2021   nex =       8

     Term   22952   22713        -        0
     Intr   23125   23045        -        0
     Intr   23255   23209        -        0
     Intr   23445   23350        -        0
     Intr   23705   23639        -        0
     Intr   24098   23940        -        0
     Intr   24254   24184        -        0
     Init   24733   24323        -        0

>4678340       /13870

   len =    1640   nex =       3

     Term    3619    3273        -        0
     Intr    3881    3700        -        0
     Init    4912    4243        -        0

>4678371       /21034

   len =     971   nex =       2

     Init  109206  109301        +        0
     Term  109446  109715        +        0

>4678371       /113114

   len =     892   nex =       2
```

```
      Init   109206   109301         +        0
      Term   109446   109693         +        0

>4678371        /98073

    len =     1111    nex =        3

      Term    41665    41333         -        0
      Intr    42181    42112         -        0
      Init    42443    42246         -        0

>4678371        /41730

    len =      440    nex =        1

      Sngl    48928    49367         +        0

>4678705        /41543

    len =     1690    nex =        3

      Init    11432    11814         +        0
      Intr    12077    12267         +        0
      Term    12387    12550         +        0

>4678705        /33399

    len =     1690    nex =        7

      Init    14667    14750         +        0
      Intr    14858    15075         +        0
      Intr    15261    15331         +        0
      Intr    15428    15529         +        0
      Intr    15646    15763         +        0
      Intr    15929    16064         +        0
      Term    16150    16346         +        0

>4678705        /125324

    len =     2171    nex =        7

      Init    16715    16857         +        0
      Intr    17191    17237         +        0
      Intr    17759    17858         +        0
      Intr    18087    18150         +        0
      Intr    18237    18353         +        0
      Intr    18453    18561         +        0
      Term    18819    18885         +        0

>4678705        /24780

    len =      791    nex =        2

      Init    25236    25351         +        0
      Term    25594    26026         +        0

>4678705        /37881

    len =     2675    nex =        5
```

```
     Term   46430   45826        -        0
     Intr   46602   46534        -        0
     Intr   46744   46682        -        0
     Intr   46896   46840        -        0
     Init   48500   47963        -        0

>4678705        /27536

    len =     281   nex =        1

     Sngl   50278   49998        -        0

>4678705        /8207

    len =    1465   nex =        1

     Sngl   51349   49885        -        0

>4678705        /15484

    len =     646   nex =        3

     Term   68931   68742        -        0
     Intr   69161   69058        -        0
     Init   69387   69316        -        0

>4680765        /19054

    len =     658   nex =        1

     Sngl  103945  104602        +        0

>4680765        /12712

    len =    1157   nex =        4

     Init    2888    2958        +        0
     Intr    3052    3180        +        0
     Intr    3402    3584        +        0
     Term    3670    4024        +        0

>4680765        /29972

    len =    1551   nex =        6

     Term    4691    4427        -        0
     Intr    4849    4793        -        0
     Intr    4976    4932        -        0
     Intr    5238    5176        -        0
     Intr    5385    5322        -        0
     Init    5977    5762        -        0

>4680765        /112559

    len =    1614   nex =        5

     Term    4691    4427        -        0
     Intr    4849    4793        -        0
     Intr    5238    4932        -        0
```

```
        Intr    5385    5322          -         0
        Init    6040    5762          -         0

    >4680765        /33173

       len =    2179    nex =       5

        Term   87951   87650          -         0
        Intr   88636   88413          -         0
        Intr   88934   88817          -         0
        Intr   89343   89032          -         0
        Init   89828   89505          -         0

    >4680765        /23348

       len =    2238    nex =       5

        Term   87951   87633          -         0
        Intr   88636   88413          -         0
        Intr   88934   88817          -         0
        Intr   89343   89032          -         0
        Init   89870   89505          -         0

    >4680765        /7057

       len =    3012    nex =       5

        Term   87951   87675          -         0
        Intr   88636   88413          -         0
        Intr   88934   88817          -         0
        Intr   89343   89032          -         0
        Init   89750   89505          -         0

    >4689466        /31988

       len =     610    nex =       1

        Sngl   44480   45086          +         0

    >4691223        /19796

       len =    1654    nex =       5

        Term  118925  118453          -         0
        Intr  119112  119052          -         0
        Intr  119318  119145          -         0
        Intr  119652  119563          -         0
        Init  120106  119970          -         0

    >4691223        /33058

       len =    1390    nex =       2

        Init  120319  120641          +         0
        Term  121277  121707          +         0

    >4691223        /7104

       len =    2650    nex =       7
```

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```
     Term    27184    26876          -          0
     Intr    27622    27443          -          0
     Intr    27988    27700          -          0
     Intr    28141    28074          -          0
     Intr    28414    28229          -          0
     Intr    28667    28503          -          0
     Init    29523    28767          -          0

 >4691223         /97866

      len =      654    nex =        3

     Term    53529    53241          -          0
     Intr    53668    53616          -          0
     Init    53894    53745          -          0

 >4691223         /118329

      len =      507    nex =        1

     Sngl    61751    62257          +          0

 >4691223         /29714

      len =      473    nex =        3

     Term    67718    67641          -          0
     Intr    67897    67808          -          0
     Init    68113    67979          -          0

 >4691223         /115554

      len =      379    nex =        1

     Sngl    72403    72781          +          0

 >4691223         /20915

      len =     3232    nex =        8

     Init    78459    78663          +          0
     Intr    79140    79266          +          0
     Intr    79400    79506          +          0
     Intr    79713    79874          +          0
     Intr    79963    80109          +          0
     Intr    80186    80380          +          0
     Intr    80488    80666          +          0
     Term    80745    80975          +          0

 >4691223         /9841

      len =      523    nex =        1

     Sngl    81514    80992          -          0

 >4699904         /21639

      len =     1521    nex =        2

     Term    29454    28950          -          0
     Init    30470    29953          -         0

 >4699904         /30530

     len =     2040    nex =         4

     Term    32070    31765          -         0
     Intr    32214    32162          -         0
     Intr    33121    33047          -         0
     Init    33255    33215          -         0

 >4699904         /37701

     len =      790    nex =         1

     Sngl    38588    37803          -         0

 >4699904         /30113

     len =      790    nex =         1

     Sngl    38588    37804          -         0

 >4699904         /105950

     len =      412    nex =         1

     Sngl    38588    38177          -         0

 >4699904         /9278

     len =      310    nex =         1

     Sngl    38588    38288          -         0

 >4699904         /8446

     len =     1770    nex =         4

     Init    82618    82845          +         0
     Intr    83226    83301          +         0
     Intr    83389    83561          +         0
     Term    84124    84387          +         0

 >4713943         /38549

     len =     2170    nex =         6

     Init    11662    11887          +         0
     Intr    12188    12265          +         0
     Intr    12354    12614          +         0
     Intr    12703    13020          +         0
     Intr    13129    13287          +         0
     Term    13447    13623          +         0

 >4713943         /19103

     len =     3190    nex =        13

     Init    18821    18995          +         0
```

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```
  Intr   19606   19673      +      0
  Intr   19753   19791      +      0
  Intr   19928   20028      +      0
  Intr   20168   20280      +      0
  Intr   20369   20468      +      0
  Intr   20547   20693      +      0
  Intr   20774   20834      +      0
  Intr   20925   21022      +      0
  Intr   21110   21252      +      0
  Intr   21361   21444      +      0
  Intr   21529   21618      +      0
  Term   21702   22007      +      0

>4713943       /10072

  len =    1233   nex =      6

  Init   20810   20834      +      0
  Intr   20925   21022      +      0
  Intr   21110   21252      +      0
  Intr   21361   21444      +      0
  Intr   21529   21618      +      0
  Term   21702   22042      +      0

>4713943       /21947

  len =    1195   nex =      2

  Init   28706   29150      +      0
  Term   29228   29900      +      0

>4713943       /16095

  len =     706   nex =      1

  Sngl   69411   70116      +      0

>4713943       /155207

  len =    1067   nex =      2

  Init   73695   73912      +      0
  Term   74344   74761      +      0

>4725940       /20383

  len =     687   nex =      1

  Sngl  106710  107396      +      0

>4725940       /6554

  len =     614   nex =      1

  Sngl  110711  111324      +      0

>4725940       /92350

  len =    1090   nex =      4
```

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```
    Term  17635   17313       -       0
    Intr  17915   17751       -       0
    Intr  18084   18006       -       0
    Init  18376   18165       -       0

>4725940        /28572

   len =     537   nex =        1

    Sngl  96158   95622       -       0

>4732167        /2296

   len =    1813   nex =        5

    Init  45294   45510       +       0
    Intr  45586   45739       +       0
    Intr  46139   46198       +       0
    Intr  46531   46591       +       0
    Term  46828   47106       +       0

>4732168        /8387

   len =     404   nex =        1

    Sngl 120482  120649       +       0

>4732168        /38543

   len =    1971   nex =        6

    Term  79492   79052       -       0
    Intr  79689   79588       -       0
    Intr  79895   79770       -       0
    Intr  80126   79992       -       0
    Intr  80429   80313       -       0
    Init  81022   80821       -       0

>4732168        /40690

   len =     670   nex =        2

    Term  80429   80357       -       0
    Init  81025   80821       -       0

>4732169        /10692

   len =     532   nex =        1

    Sngl  52688   52157       -       0

>4732169        /25430

   len =     550   nex =        1

    Sngl  52692   52146       -       0

>4733952        /37506

   len =    2578   nex =        7
```

```
     Init  117152  117890         +       0
     Intr  117977  118056         +       0
     Intr  118424  118501         +       0
     Intr  118902  118997         +       0
     Intr  119102  119227         +       0
     Intr  119304  119369         +       0
     Term  119460  119729         +       0

>4733952        /11975

    len =     1396   nex =       2

     Init     2301     2629       +       0
     Term     3230     3696       +       0

>4733952        /10295

    len =     1244   nex =       3

     Init     4419     5051       +       0
     Intr     5122     5416       +       0
     Term     5495     5662       +       0

>4733952        /109289

    len =     1232   nex =       3

     Init     4431     5051       +       0
     Intr     5122     5416       +       0
     Term     5495     5662       +       0

>4733952        /36621

    len =     1918   nex =       6

     Term    86725    86366       -       0
     Intr    87006    86815       -       0
     Intr    87271    87194       -       0
     Intr    87467    87359       -       0
     Intr    87678    87564       -       0
     Init    88281    87885       -       0

>4733952        /28602

    len =      599   nex =       1

     Sngl    97194    97792       +       0

>4733952        /32795

    len =      719   nex =       1

     Sngl    97257    97975       +       0

>4733953        /106946

    len =      473   nex =       1

     Sngl   118125   117810       -       0
```

```
>4733953        /29385

   len =     2099    nex =        6

     Term   10729    10095          -         0
     Intr   10883    10802          -         0
     Intr   11125    11042          -         0
     Intr   11384    11217          -         0
     Intr   11617    11461          -         0
     Init   12193    11723          -         0

>4733953        /15169

   len =     1239    nex =        2

     Term   16338    16088          -         0
     Init   17326    17040          -         0

>4733953        /2629

   len =     1270    nex =        1

     Sngl   16338    16093          -         0

>4733953        /39404

   len =      379    nex =        1

     Sngl   17961    17583          -         0

>4733953        /19759

   len =     2110    nex =        3

     Init   20860    21383          +         0
     Intr   21459    21893          +         0
     Term   22639    22966          +         0

>4733953        /30753

   len =     1171    nex =        4

     Term   60229    59859          -         0
     Intr   60602    60530          -         0
     Intr   60835    60709          -         0
     Init   61029    60968          -         0

>4733953        /37277

   len =     2830    nex =        9

     Init   61852    62588          +         0
     Intr   62673    62871          +         0
     Intr   62958    63050          +         0
     Intr   63137    63265          +         0
     Intr   63340    63393          +         0
     Intr   63487    63564          +         0
     Intr   63686    63748          +         0
     Intr   63865    63977          +         0
```

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Reference No. 2750-942P

```
    Term    64059   64359         +        0

>4733953        /101924

    len =      730   nex =        3

    Init        73     181        +        0
    Intr       262     357        +        0
    Term       434     801        +        0

>4733953        /32143

    len =     1905   nex =        4

    Term     97964   97577        -        0
    Intr     98361   98053        -        0
    Intr     98605   98433        -        0
    Init     99481   99057        -        0

>4733957        /125929

    len =      970   nex =        2

    Term      7176    6924        -        0
    Init      7349    7257        -        0

>4733984        /26824

    len =     2050   nex =        3

    Init     47232   47598        +        0
    Intr     47718   47928        +        0
    Term     48007   48272        +        0

>4733984        /29669

    len =     1938   nex =        3

    Init     47232   47598        +        0
    Intr     47718   47928        +        0
    Term     48007   48229        +        0

>4734003        /93895

    len =      533   nex =        1

    Sngl     20795   21327        +        0

>4734003        /32391

    len =     2026   nex =        6

    Init     45023   45326        +        0
    Intr     45415   45959        +        0
    Intr     46054   46140        +        0
    Intr     46221   46277        +        0
    Intr     46354   46798        +        0
    Term     46863   47048        +        0

>4734003        /9500
```

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```
    len =     802   nex =       2

     Term   70600   70084        -        0
     Init   70885   70675        -        0

>4734003        /28779

    len =    1210   nex =       5

     Term   69956   69706        -        0
     Intr   70115   70043        -        0
     Intr   70462   70257        -        0
     Intr   70600   70537        -        0
     Init   70912   70675        -        0

>4753195        /5767

    len =     895   nex =       2

     Term  102330  101848        -        0
     Init  102742  102437        -        0

>4753195        /20865

    len =    1902   nex =       5

     Term   24230   23691        -        0
     Intr   24471   24314        -        0
     Intr   24713   24567        -        0
     Intr   25027   24796        -        0
     Init   25592   25383        -        0

>4753195        /7837

    len =    2021   nex =       9

     Init   41032   41313        +        0
     Intr   41510   41597        +        0
     Intr   41693   41806        +        0
     Intr   41892   41974        +        0
     Intr   42064   42178        +        0
     Intr   42268   42359        +        0
     Intr   42443   42508        +        0
     Intr   42608   42701        +        0
     Term   42827   43052        +        0

>4753645        /42927

    len =     889   nex =       1

     Sngl   15300   14412        -        0

>4753645        /748

    len =     912   nex =       1

     Sngl   15333   14422        -        0

>4753645        /11649
```

```
    len =      778   nex =        1

      Sngl   20284   19507         -         0

>4753645        /18082

    len =     1035   nex =        3

      Term   25637   25286         -         0
      Intr   26018   25864         -         0
      Init   26320   26110         -         0

>4753645        /4311

    len =      991   nex =        4

      Term   31713   31426         -         0
      Intr   31904   31800         -         0
      Intr   32064   32003         -         0
      Init   32416   32195         -         0

>4753645        /121013

    len =     1018   nex =        3

      Term   33198   32799         -         0
      Intr   33586   33527         -         0
      Init   33816   33685         -         0

>4753645        /20847

    len =     1904   nex =        8

      Term   41741   41372         -         0
      Intr   42028   41836         -         0
      Intr   42227   42105         -         0
      Intr   42440   42301         -         0
      Intr   42595   42527         -         0
      Intr   42881   42686         -         0
      Intr   43051   42977         -         0
      Init   43275   43144         -         0

>4755178        /7413

    len =      550   nex =        1

      Sngl   23401   23949         +         0

>4755179        /37863

    len =     2208   nex =        4

      Term   38083   37560         -         0
      Intr   38382   38183         -         0
      Intr   38861   38468         -         0
      Init   39767   39548         -         0

>4755179        /33047
```

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```
        len =     477    nex =       1

         Sngl   45523   45047          -         0

>4755179        /13002

        len =    1676    nex =       2

         Init   50115   50339          +         0
         Term   50656   51087          +         0

>4755179        /125387

        len =     774    nex =       2

         Init   50164   50339          +         0
         Term   50656   50937          +         0

>4755179        /40183

        len =    2251    nex =       7

         Term   73491   73290          -         0
         Intr   73894   73775          -         0
         Intr   74166   73981          -         0
         Intr   74402   74263          -         0
         Intr   75048   74800          -         0
         Intr   75246   75129          -         0
         Init   75540   75409          -         0

>4755179        /4933

        len =    1523    nex =       0

>4755179        /40305

        len =    1342    nex =       1

         Sngl   82160   83501          +         0

>4755179        /112223

        len =     279    nex =       1

         Sngl   86044   85766          -         0

>4755179        /230

        len =    2782    nex =       8

         Term   90030   89591          -         0
         Intr   90265   90182          -         0
         Intr   90522   90366          -         0
         Intr   90939   90674          -         0
         Intr   91320   91048          -         0
         Intr   91530   91419          -         0
         Intr   91829   91610          -         0
         Init   92372   91910          -         0

>4755179        /113514
```

09755920 .061601

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```
len =     2378    nex =        6

 Term     7459     7240          -         0
 Intr     7721     7576          -         0
 Intr     7889     7809          -         0
 Intr     8124     7978          -         0
 Intr     8629     8530          -         0
 Init     9601     9467          -         0

>4755185        /27930

len =      861    nex =        3

 Term   104138   103879          -         0
 Intr   104520   104451          -         0
 Init   104739   104586          -         0

>4755185        /4047

len =      885    nex =        1

 Sngl   104763   103879          -         0
>4755185        /5198

len =      910    nex =        3

 Term   104138   103878          -         0
 Intr   104520   104451          -         0
 Init   104780   104586          -         0

>4755185        /99800

len =      996    nex =        0

>4755185        /33377

len =     1606    nex =        1

 Sngl    89482    90543          +         0

>4757388        /34183

len =      996    nex =        3

 Term      686       30          -         0
 Intr      828      758          -         0
 Init     1025      911          -         0

>4757388        /20852

len =     1221    nex =        2

 Init    26560    26673          +         0
 Term    26774    27780          +         0

>4757390        /37617

len =     2548    nex =        3
```

```
      Term   13877   13660        -       0
      Intr   14019   13968        -       0
      Init   16207   15422        -       0

>4757390       /109272

    len =     528    nex =       1

      Sngl   37853   38380        +       0

>4757390       /100074

    len =     519    nex =       1

      Sngl   37862   38380        +       0

>4757390       /103168

    len =    1856    nex =       4

      Init   41052   41145        +       0
      Intr   41501   41594        +       0
      Intr   42124   42386        +       0
      Term   42471   42907        +       0

>4757392       /2102

    len =    3220    nex =      13

      Term   18611   18194        -       0
      Intr   18807   18736        -       0
      Intr   19090   18977        -       0
      Intr   19281   19168        -       0
      Intr   19487   19387        -       0
      Intr   19668   19590        -       0
      Intr   19823   19749        -       0
      Intr   19989   19927        -       0
      Intr   20445   20365        -       0
      Intr   20669   20544        -       0
      Intr   21040   20916        -       0
      Intr   21231   21126        -       0
      Init   21413   21349        -       0

>4757392       /31656

    len =     970    nex =       3

      Term   28052   27639        -       0
      Intr   28365   28148        -       0
      Init   28605   28452        -       0

>4757392       /96588

    len =     590    nex =       2

      Term   41513   41072        -       0
      Init   41654   41598        -       0

>4757392       /13932
```

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```
   len =     1822    nex =        5

    Term    48918    48477         -        0
    Intr    49253    49006         -        0
    Intr    49533    49327         -        0
    Intr    49777    49615         -        0
    Init    50298    50008         -        0

>4757392        /36559

   len =     1821    nex =        4

    Term    48918    48478         -        0
    Intr    49253    49006         -        0
    Intr    49533    49327         -        0
    Init    49777    49615         -        0

>4757392        /2204

   len =      550    nex =        1

    Sngl     5387     4851         -        0

>4757392        /9425

   len =      579    nex =        1

    Sngl    57089    57667         +        0

>4757392        /111154

   len =     1092    nex =        0

>4757392        /26899

   len =     2182    nex =        4

    Init    73097    73405         +        0
    Intr    73958    74660         +        0
    Intr    74754    74910         +        0
    Term    74995    75278         +        0

>4757392        /12456

   len =     1995    nex =        4

    Init    73287    73405         +        0
    Intr    73958    74660         +        0
    Intr    74754    74910         +        0
    Term    74995    75281         +        0

>4757392        /35237

   len =      254    nex =        1

    Sngl    75025    75278         +        0

>4757392        /16548

   len =     1845    nex =        5
```

```
     Term   85494   84859         -        0
     Intr   85695   85618         -        0
     Intr   86164   86108         -        0
     Intr   86314   86260         -        0
     Init   86703   86408         -        0

>4757395       /15932

    len =     801   nex =       2

     Term   42705   42137         -        0
     Init   42937   42787         -        0

>4757395       /13267

    len =    1911   nex =       4

     Init   53364   54057         +        0
     Intr   54543   54725         +        0
     Intr   54857   55024         +        0
     Term   55121   55274         +        0

>4757395       /118150

    len =    1918   nex =       3

     Init   55805   56142         +        0
     Intr   56228   56443         +        0
     Term   57409   57722         +        0

>4757396       /4410

    len =     471   nex =       1

     Sngl   15964   16434         +        0

>4757396       /115489

    len =     459   nex =       1

     Sngl   15976   16434         +        0

>4757399       /115876

    len =     341   nex =       1

     Sngl    1954    2294         +        0

>4757400       /11763

    len =    1275   nex =       2

     Term   74376   74009         -        0
     Init   75283   74843         -        0

>4757400       /8330

    len =    2249   nex =       8
```

```
     Term   80687   80160        -        0
     Intr   80926   80866        -        0
     Intr   81202   81027        -        0
     Intr   81363   81306        -        0
     Intr   81868   81764        -        0
     Intr   82022   81965        -        0
     Intr   82194   82108        -        0
     Init   82408   82279        -        0

>4757401       /924

    len =     1438   nex =       3

     Term   13076   12445        -        0
     Intr   13565   13169        -        0
     Init   13882   13663        -        0

>4757401       /103058

    len =      550   nex =       1

     Sngl   22797   22249        -        0

>4757401       /115850

    len =      614   nex =       1

     Sngl   26499   25886        -        0

>4757401       /27707

    len =      716   nex =       2

     Term   27638   27345        -        0
     Init   28060   27979        -        0

>4757401       /124616

    len =      645   nex =       1

     Sngl   32214   31570        -        0

>4757401       /27482

    len =     2410   nex =       6

     Init   75050   75395        +        0
     Intr   75597   75744        +        0
     Intr   75850   76110        +        0
     Intr   76313   76357        +        0
     Intr   76564   76732        +        0
     Term   76891   77454        +        0

>4757403       /13058

    len =     1510   nex =       7

     Term   15873   15797        -        0
     Intr   16028   15960        -        0
     Intr   16200   16113        -        0
```

```
      Intr   16374    16279          -         0
      Intr   16632    16519          -         0
      Intr   16870    16737          -         0
      Init   17303    16971          -         0

>4757403       /38239

     len =     2056    nex =       7

      Term   15873    15307          -         0
      Intr   16028    15960          -         0
      Intr   16200    16113          -         0
      Intr   16374    16279          -         0
      Intr   16632    16519          -         0
      Intr   16870    16737          -         0
      Init   17362    16971          -         0

>4757403       /19537

     len =     1552    nex =       4

      Term   19050    18584          -         0
      Intr   19389    19285          -         0
      Intr   19620    19513          -         0
      Init   20135    19978          -         0

>4757403       /24666

     len =     1611    nex =       2

      Init   24264    25305          +         0
      Term   25397    25874          +         0

>4757403       /39358

     len =     2877    nex =       8

      Term   26486    25989          -         0
      Intr   26777    26718          -         0
      Intr   26988    26867          -         0
      Intr   27249    27072          -         0
      Intr   27624    27359          -         0
      Intr   28030    27742          -         0
      Intr   28252    28115          -         0
      Init   28406    28339          -         0

>4757403       /39534

     len =      554    nex =       1

      Sngl   32923    32370          -         0

>4757403       /7337

     len =      503    nex =       1

      Sngl   35283    34781          -         0

>4757403       /40618
```

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```
 len =     1967    nex =       6

  Init   51540    51679          +         0
  Intr   51794    51843          +         0
  Intr   52329    52391          +         0
  Intr   52696    52786          +         0
  Intr   52894    52998          +         0
  Term   53300    53506          +         0

>4757403        /38488

 len =      610    nex =       1

  Sngl   77818    78427          +         0

>4757404        /103083

 len =      143    nex =       1

  Sngl   32868    33010          +         0

>4757405        /36106

 len =     2269    nex =       7

  Term    8267     7900          -         0
  Intr    8590     8359          -         0
  Intr    8829     8685          -         0
  Intr    9156     8911          -         0
  Intr    9449     9249          -         0
  Intr    9730     9637          -         0
  Init   10168     9818          -         0

>4757405        /20648

 len =      518    nex =       1

  Sngl   39529    39012          -         0

>4757405        /94317

 len =      397    nex =       1

  Sngl   41006    40610          -         0

>4757405        /100878

 len =     1572    nex =       7

  Term   45130    44797          -         0
  Intr   45395    45330          -         0
  Intr   45558    45474          -         0
  Intr   45726    45633          -         0
  Intr   45884    45827          -         0
  Intr   46200    46160          -         0
  Init   46368    46277          -         0

>4757405        /5385

 len =      599    nex =       1
```

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```
     Sngl   47192   46605        -       0

>4757405        /40773

    len =     598   nex =       1

     Sngl   47192   46606        -       0

>4757405        /30812

    len =    1544   nex =       6

     Term   52068   51817        -       0
     Intr   52294   52205        -       0
     Intr   52456   52409        -       0
     Intr   52724   52555        -       0
     Intr   53078   53000        -       0
     Init   53360   53196        -       0

>4757405        /16619

    len =     761   nex =       2

     Init   56271   56389        +       0
     Term   56759   57031        +       0

>4757405        /3049

    len =    2274   nex =       5

     Init   56278   57300        +       0
     Intr   57384   57484        +       0
     Intr   57586   57669        +       0
     Intr   57761   57871        +       0
     Term   58094   58551        +       0

>4757406        /19244

    len =    1177   nex =       2

     Init   25061   25439        +       0
     Term   25638   26237        +       0

>4757406        /35710

    len =    1589   nex =       8

     Init   31143   31329        +       0
     Intr   31449   31591        +       0
     Intr   31679   31786        +       0
     Intr   31888   31932        +       0
     Intr   32002   32067        +       0
     Intr   32152   32212        +       0
     Intr   32296   32342        +       0
     Term   32424   32731        +       0

>4757406        /97340

    len =    2530   nex =       5
```

```
     Init    33489    33681          +         0
     Intr    34354    34432          +         0
     Intr    34531    34598          +         0
     Intr    34773    34839          +         0
     Term    35246    35497          +         0

>4757407          /145523

   len =      522    nex =        2

     Init    14432    14663          +         0
     Term    14777    14953          +         0

>4757407          /16944

   len =     1228    nex =        5

     Term    35221    34886          -         0
     Intr    35427    35369          -         0
     Intr    35569    35507          -         0
     Intr    35739    35676          -         0
     Init    36113    35815          -         0

>4757407          /18624

   len =      562    nex =        1

     Sngl    42900    43461          +         0

>4757407          /19127

   len =     2025    nex =        5

     Term    44665    44410          -         0
     Intr    44838    44752          -         0
     Intr    45182    44922          -         0
     Intr    45743    45338          -         0
     Init    46434    45860          -         0

>4757407          /41161

   len =     1106    nex =        3

     Init     5273     5534          +         0
     Intr     5627     5929          +         0
     Term     6019     6378          +         0

>4757407          /11077

   len =      466    nex =        1

     Sngl     6030     6495          +         0

>4757407          /111727

   len =     3141    nex =        5

     Init    75926    76044          +         0
     Intr    76156    76326          +         0
```

```
         Intr    76407    76463          +         0
         Intr    76541    76605          +         0
         Term    76716    76840          +         0

      >4757407         /12484

        len =     1893    nex =        4

         Term    77674    77314          -         0
         Intr    77970    77850          -         0
         Intr    78287    78047          -         0
         Init    79206    78928          -         0

      >4757407         /26596

        len =     1953    nex =        5

         Term    82383    82097          -         0
         Intr    82758    82519          -         0
         Intr    83472    83378          -         0
         Intr    83776    83689          -         0
         Init    84049    84002          -         0

      >4757409         /6166

        len =     2028    nex =        3

         Init     2893     3040          +         0
         Intr     3127     3389          +         0
         Term     3462     4256          +         0

      >4757409         /17962

        len =      790    nex =        2

         Term     4534     4275          -         0
         Init     5064     4726          -         0

      >4757410         /13767

        len =     2170    nex =        5

         Init    11323    11581          +         0
         Intr    11799    11940          +         0
         Intr    12035    12088          +         0
         Intr    12453    12544          +         0
         Term    12656    12882          +         0

      >4757410         /104278

        len =      970    nex =        1

         Sngl    14335    13368          -         0

      >4757410         /5180

        len =      626    nex =        2

         Init    37202    37397          +         0
         Term    37493    37827          +         0
```

```
>4757410       /92670

   len =    1849   nex =       2

    Term   42000   41613         -         0
    Init   43461   43210         -         0

>4757410       /29464

   len =     895   nex =       1

    Sngl   50057   50951         +         0

>4757410       /3819

   len =    2093   nex =       2

    Init   59901   60514         +         0
    Term   60601   60838         +         0

>4757410       /34479

   len =    1298   nex =       3

    Init   59369   59822         +         0
    Intr   59901   60514         +         0
    Term   60601   60666         +         0

>4757410       /23740

   len =    1303   nex =       3

    Init   59614   59822         +         0
    Intr   59901   60514         +         0
    Term   60601   60916         +         0

>4757410       /36248

   len =     616   nex =       2

    Init   60322   60514         +         0
    Term   60601   60918         +         0

>4757410       /101693

   len =    2146   nex =       6

    Init   67945   68152         +         0
    Intr   68845   68971         +         0
    Intr   69127   69273         +         0
    Intr   69364   69444         +         0
    Intr   69538   69683         +         0
    Term   69773   70080         +         0

>4757410       /123200

   len =     284   nex =       1

    Sngl   73051   72768         -         0
```

```
>4757411       /99062

   len =     348   nex =       1

    Sngl   14302   14649        +        0

>4757411       /38286

   len =    2413   nex =       5

    Init   35325   35620        +        0
    Intr   36178   36431        +        0
    Intr   36689   36824        +        0
    Intr   37137   37198        +        0
    Term   37290   37737        +        0

>4757411       /143077

   len =     774   nex =       3

    Term   48735   48361        -        0
    Intr   48922   48839        -        0
    Init   49134   48982        -        0

>4757413       /39613

   len =    1930   nex =       6

    Term   10486   10044        -        0
    Intr   10673   10581        -        0
    Intr   10937   10794        -        0
    Intr   11196   11110        -        0
    Intr   11493   11268        -        0
    Init   11973   11691        -        0

>4757413       /37104

   len =    1352   nex =       2

    Init   37253   37571        +        0
    Term   37707   38604        +        0

>4757413       /37513

   len =    1375   nex =       2

    Init   37253   37571        +        0
    Term   37707   38627        +        0

>4757413       /9683

   len =    1290   nex =       1

    Sngl   40182   39244        -        0

>4757414       /94738

   len =     731   nex =       1
```

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```
    Sngl   15443   14713          -        0

>4757414       /1185

    len =    1812   nex =       2

    Init   21364   21435          +        0
    Term   21537   22038          +        0

>4757414       /113827

    len =     443   nex =       1

    Sngl   35589   36031          +        0

>4757414       /117748

    len =     615   nex =       2

    Init   41281   41606          +        0
    Term   41845   41895          +        0

>4757414       /6463

    len =    1403   nex =       3

    Init   41281   41606          +        0
    Intr   41845   41895          +        0
    Term   42583   42683          +        0

>4757414       /23733

    len =    1161   nex =       1

    Sngl   43908   42748          -        0

>4757414       /32274

    len =    1690   nex =       5

    Init   44451   44575          +        0
    Intr   44666   44762          +        0
    Intr   44860   44969          +        0
    Intr   45357   45431          +        0
    Term   45541   45739          +        0

>4757414       /17361

    len =     167   nex =       1

    Sngl   67827   67661          -        0

>4757414       /21721

    len =    4353   nex =      15

    Init   79949   80134          +        0
    Intr   80262   80364          +        0
    Intr   80481   80545          +        0
    Intr   80685   80760          +        0
```

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```
      Intr   81080   81144        +        0
      Intr   81467   81590        +        0
      Intr   81701   81826        +        0
      Intr   81943   81993        +        0
      Intr   82107   82175        +        0
      Intr   82394   82502        +        0
      Intr   82646   82725        +        0
      Intr   82828   82910        +        0
      Intr   82992   83037        +        0
      Intr   83119   83293        +        0
      Term   83381   83643        +        0

>4757415        /43010

     len =     2179    nex =        5

      Init     110      480       +        0
      Intr     969     1054       +        0
      Intr    1131     1436       +        0
      Intr    1538     1621       +        0
      Term    1706     2288       +        0

>4757415        /33108

     len =     1990    nex =        5

      Term   18813   18525        -        0
      Intr   19074   18908        -        0
      Intr   19639   19580        -        0
      Intr   19870   19724        -        0
      Init   20507   20200        -        0

>4757415        /35741

     len =     2319    nex =        7

      Term   21262   20642        -        0
      Intr   21516   21346        -        0
      Intr   21769   21603        -        0
      Intr   21961   21868        -        0
      Intr   22248   22040        -        0
      Intr   22485   22334        -        0
      Init   22960   22702        -        0

>4757415        /94723

     len =      910    nex =        3

      Term    8317     8042       -        0
      Intr    8742     8423       -        0
      Init    8946     8856       -        0

>4757417        /12993

     len =     2378    nex =        8

      Init   57794   57865        +        0
      Intr   57948   58085        +        0
      Intr   58174   58272        +        0
      Intr   58373   58445        +        0
     Intr   58911   58999         +       0
     Intr   59128   59211         +       0
     Intr   59310   59369         +       0
     Term   59441   59775         +       0

>4757417       /40272

    len =    2254   nex =       8

     Init   57533   57711         +       0
     Intr   57794   57865         +       0
     Intr   57948   58085         +       0
     Intr   58174   58272         +       0
     Intr   58373   58445         +       0
     Intr   59128   59211         +       0
     Intr   59310   59369         +       0
     Term   59441   59786         +       0

>4757660       /34549

    len =    2683   nex =       8

     Init   17650   17772         +       0
     Intr   18075   18155         +       0
     Intr   18244   18332         +       0
     Intr   18448   18522         +       0
     Intr   18662   18726         +       0
     Intr   18812   18883         +       0
     Intr   18993   19430         +       0
     Term   19524   20051         +       0

>4757660       /34984

    len =    1570   nex =       6

     Init   23805   24076         +       0
     Intr   24164   24212         +       0
     Intr   24293   24419         +       0
     Intr   24566   24636         +       0
     Intr   24733   24805         +       0
     Term   25086   25372         +       0

>4757660       /18595

    len =     757   nex =       1

     Sngl    3045    3801         +       0

>4757660       /6179

    len =    1773   nex =       5

     Term   38593   38287         -       0
     Intr   39063   38802         -       0
     Intr   39389   39159         -       0
     Intr   39630   39497         -       0
     Init   40059   39789         -       0

>4757660       /31508
```

```
      len =     1017    nex =        2

       Term   41278    40970          -          0
       Init   41687    41426          -          0

  >4757660        /295

      len =     1534    nex =        5

       Term   41278    41051          -          0
       Intr   41687    41426          -          0
       Intr   42011    41778          -          0
       Intr   42219    42089          -          0
       Init   42584    42322          -          0

  >4757660        /24456

      len =     1006    nex =        2

       Term   45205    44865          -          0
       Init   45691    45430          -          0

  >4757660        /13876

      len =     1838    nex =        5

       Term   45205    44861          -          0
       Intr   45691    45430          -          0
       Intr   46019    45783          -          0
       Intr   46265    46135          -          0
       Init   46698    46390          -          0

  >4757660        /30015

      len =     1879    nex =        5

       Term   47388    47089          -          0
       Intr   47741    47486          -          0
       Intr   48105    47875          -          0
       Intr   48353    48223          -          0
       Init   48967    48677          -          0

  >4757660        /25120

      len =     1990    nex =        5

       Term   47388    47089          -          0
       Intr   47741    47486          -          0
       Intr   48105    47875          -          0
       Intr   48353    48223          -          0
       Init   49075    48677          -          0

  >4757660        /34896

      len =     1999    nex =        5

       Term   54992    54648          -          0
       Intr   55353    55098          -          0
       Intr   55706    55473          -          0
       Intr   55926    55796          -          0
```

```
    Init   56646   56224        -        0

>4757660       /13762

    len =     1939   nex =       7

    Term   69818   69378        -        0
    Intr   69980   69915        -        0
    Intr   70123   70059        -        0
    Intr   70269   70206        -        0
    Intr   70491   70382        -        0
    Intr   70666   70579        -        0
    Init   71316   71035        -        0

>4757660       /2663

    len =      589   nex =       1

    Sngl   85423   84835        -        0

>4757660       /143795

    len =      550   nex =       1

    Sngl    9114    8573        -        0

>4757661       /33846

    len =      998   nex =       0

>4757661       /18244

    len =     1550   nex =       4

    Init   20501   20937        +        0
    Intr   21063   21205        +        0
    Intr   21578   21697        +        0
    Term   21792   22050        +        0

>4757661       /12689

    len =     1820   nex =       3

    Term   52653   52143        -        0
    Intr   53031   52754        -        0
    Init   53962   53118        -        0

>4757661       /37307

    len =     1947   nex =       5

    Init   79034   79330        +        0
    Intr   79685   79897        +        0
    Intr   79984   80132        +        0
    Intr   80266   80542        +        0
    Term   80634   80980        +        0

>4757661       /38874

    len =     1550   nex =       3
```

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```
     Init    88004    88300          +         0
     Intr    88888    89103          +         0
     Term    89479    89553          +         0

>4757661        /29823

    len =     1270    nex =        2

     Term    95428    95219          -         0
     Init    96487    96281          -         0

>4757662        /151497

    len =      374    nex =        1

     Sngl   105041   105406          +         0

>4757662        /33355

    len =      764    nex =        1

     Sngl   105041   105804          +         0

>4757662        /10525

    len =      959    nex =        2

     Term   107176   106447          -         0
     Init   107405   107251          -         0

>4757662        /40979

    len =     2536    nex =        1

     Sngl    11058    12235          +         0

>4757662        /3858

    len =      883    nex =        2

     Term   127589   127180          -         0
     Init   128062   127787          -         0

>4757662        /25577

    len =      641    nex =        1

     Sngl    15911    16551          +         0

>4757662        /13756

    len =     2333    nex =        6

     Init    26705    26948          +         0
     Intr    27713    27814          +         0
     Intr    27911    28038          +         0
     Intr    28342    28470          +         0
     Intr    28565    28632          +         0
     Term    28747    29037          +         0
```

```
>4757662        /19079

   len =     1931    nex =        4

    Term   35291   34651        -        0
    Intr   35971   35763        -        0
    Intr   36176   36076        -        0
    Init   36581   36264        -        0

>4757662        /206217

   len =      475    nex =        1

    Sngl   51723   51806        +        0

>4757662        /41103

   len =     2145    nex =        5

    Term   55687   54683        -        0
    Intr   55901   55794        -        0
    Intr   56205   56003        -        0
    Intr   56423   56280        -        0
    Init   56827   56682        -        0

>4757662        /10861

   len =     1789    nex =        5

    Term   73379   73086        -        0
    Intr   73578   73468        -        0
    Intr   73781   73664        -        0
    Intr   73905   73869        -        0
    Init   74874   74703        -        0

>4757662        /12880

   len =     4180    nex =       16

    Term   75805   75445        -        0
    Intr   76039   75900        -        0
    Intr   76358   76118        -        0
    Intr   76502   76452        -        0
    Intr   76821   76596        -        0
    Intr   77212   77142        -        0
    Intr   77417   77298        -        0
    Intr   77706   77512        -        0
    Intr   77866   77802        -        0
    Intr   78048   77958        -        0
    Intr   78257   78196        -        0
    Intr   78415   78365        -        0
    Intr   78718   78617        -        0
    Intr   78917   78810        -        0
    Intr   79200   79137        -        0
    Init   79624   79320        -        0

>4757662        /8333

   len =      587    nex =        1
```

```
     Sngl    97501    97352          -          0

  >4757678         /17521

    len =      808    nex =        1

     Sngl     1711      904          -          0

  >4757678         /1241

    len =     1888    nex =        8

     Init    53235    53440          +          0
     Intr    53822    53963          +          0
     Intr    54052    54113          +          0
     Intr    54206    54317          +          0
     Intr    54450    54543          +          0
     Intr    54631    54700          +          0
     Intr    54792    54930          +          0
     Term    55025    55122          +          0

  >4757678         /6028

    len =      675    nex =        1

     Sngl    62812    62712          -          0

  >4757678         /96337

    len =      910    nex =        1

     Sngl    85389    85713          +          0

  >4757686         /24090

    len =     1474    nex =        6

     Term    49322    49010          -          0
     Intr    49490    49428          -          0
     Intr    49666    49586          -          0
     Intr    49814    49770          -          0
     Intr    50155    50066          -          0
     Init    50294    50238          -          0

  >4757688         /117787

    len =     1051    nex =        4

     Term    32809    32597          -          0
     Intr    33059    32975          -          0
     Intr    33518    33485          -          0
     Init    33647    33604          -          0

  >4760247         /123742

    len =      929    nex =        5

     Term    16228    15990          -          0
     Intr    16403    16313          -          0
```

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```
    Intr   16601   16514          -        0
    Intr   16705   16680          -        0
    Init   16918   16792          -        0

>4760247         /7361

   len =     2251    nex =        6

    Init     1746     2458        +        0
    Intr     2735     2837        +        0
    Intr     2918     3021        +        0
    Intr     3285     3376        +        0
    Intr     3462     3559        +        0
    Term     3647     3996        +        0

>4760247         /102245

   len =      465    nex =        1

    Sngl     1766     2230        +        0

>4760247         /16412

   len =     2700    nex =       12

    Term   16228   15990          -        0
    Intr   16403   16313          -        0
    Intr   16601   16514          -        0
    Intr   17018   16792          -        0
    Intr   17212   17097          -        0
    Intr   17537   17282          -        0
    Intr   17721   17634          -        0
    Intr   17870   17799          -        0
    Intr   18036   17928          -        0
    Intr   18186   18128          -        0
    Intr   18460   18397          -        0
    Init   18689   18561          -        0

>4760247         /36952

   len =     2304    nex =        5

    Term     6330     6048        -        0
    Intr     7503     7393        -        0
    Intr     7682     7577        -        0
    Intr     7899     7771        -        0
    Init     8351     7975        -        0

>4760411         /33299

   len =     2250    nex =        9

    Term   21949   21710          -        0
    Intr   22229   22137          -        0
    Intr   22409   22356          -        0
    Intr   22653   22513          -        0
    Intr   22805   22740          -        0
    Intr   23090   22889          -        0
    Intr   23430   23376          -        0
    Intr   23651   23590          -        0
    Init   23959   23734         -         0

>4760411        /16491

    len =     1975    nex =        4

    Init    4186    4655         +         0
    Intr    4887    5035         +         0
    Intr    5129    5239         +         0
    Term    5334    6160         +         0

>4760411        /120348

    len =      653    nex =        1

    Sngl   45700   45048         -         0

>4760411        /31883

    len =      152    nex =        1

    Sngl    6057    6208         +         0

>4760411        /36997

    len =     2050    nex =        8

    Term   67189   66970         -         0
    Intr   67456   67326         -         0
    Intr   67716   67588         -         0
    Intr   67946   67800         -         0
    Intr   68253   68062         -         0
    Intr   68522   68412         -         0
    Intr   68705   68610         -         0
    Init   69013   68843         -         0

>4760411        /30022

    len =     3315    nex =        6

    Term   73699   73294         -         0
    Intr   74021   73925         -         0
    Intr   74270   74140         -         0
    Intr   74416   74358         -         0
    Intr   76009   75544         -         0
    Init   76608   76282         -         0

>4760411        /38193

    len =     1474    nex =        1

    Sngl   77039   78512         +         0

>4761801        /93707

    len =      619    nex =        1

    Sngl  143737  143119         -         0

>4761801        /115966
```

```
   len =      649   nex =        1

    Sngl  151005  151653          +        0

>4761801        /10618

   len =     1736   nex =        5

    Init  168932  169123          +        0
    Intr  169266  169370          +        0
    Intr  169478  169788          +        0
    Intr  169866  170051          +        0
    Term  170355  170667          +        0

>4761801        /30044

   len =     2558   nex =        5

    Init    26588   27075         +        0
    Intr    27771   27904         +        0
    Intr    28035   28462         +        0
    Intr    28545   28676         +        0
    Term    28863   29145         +        0

>4761801        /103581

   len =      924   nex =        1

    Sngl    40802   39879         -        0

>4761801        /96744

   len =     1859   nex =        6

    Init    46090   46263         +        0
    Intr    46495   46564         +        0
    Intr    46666   46725         +        0
    Intr    46946   47060         +        0
    Intr    47328   47443         +        0
    Term    47528   47948         +        0

>4761801        /27650

   len =      643   nex =        2

    Init    47359   47443         +        0
    Term    47528   47991         +        0

>4761801        /34620

   len =     4450   nex =       14

    Term    83154   82922         -        0
    Intr    83294   83234         -        0
    Intr    83538   83451         -        0
    Intr    83672   83607         -        0
    Intr    84088   83988         -        0
    Intr    84594   84487         -        0
    Intr    85379   85289         -        0
```

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```
     Intr    85690    85604          -         0
     Intr    85964    85841          -         0
     Intr    86148    86057          -         0
     Intr    86290    86229          -         0
     Intr    86558    86461          -         0
     Intr    87054    86904          -         0
     Init    87364    87194          -         0

>4775266        /119956

    len =      1411    nex =        2

     Init    17383    17754          +         0
     Term    18006    18793          +         0

>4775266        /20805

    len =      2816    nex =        9

     Init    19777    19920          +         0
     Intr    20186    20444          +         0
     Intr    20536    20742          +         0
     Intr    20819    21069          +         0
     Intr    21162    21486          +         0
     Intr    21579    21842          +         0
     Intr    21927    22048          +         0
     Intr    22133    22252          +         0
     Term    22327    22592          +         0

>4775266        /20161

    len =       557    nex =        1

     Sngl    25109    24553          -         0

>4775266        /33023

    len =      1956    nex =        4

     Term    27412    26849          -         0
     Intr    27825    27714          -         0
     Intr    28452    28152          -         0
     Init    28804    28549          -         0

>4775266        /10077

    len =       715    nex =        2

     Term    38914    38632          -         0
     Init    39346    39105          -         0

>4775266        /18040

    len =       801    nex =        1

     Sngl    44171    44971          +         0

>4775266        /7221

    len =      1630    nex =        6
```

Reference No. 2750-942P

```
     Term     4598     4139          -        0
     Intr     4777     4686          -        0
     Intr     4950     4865          -        0
     Intr     5215     5035          -        0
     Intr     5520     5300          -        0
     Init     5766     5592          -        0

>4803878          /4897

    len  =      574    nex  =         1

     Sngl    22484    21911          -        0

>4803878          /39544

    len  =     1063    nex  =         3

     Term    38379    37933          -        0
     Intr    38854    38542          -        0
     Init    38995    38931          -        0

>4803878          /37896

    len  =     1436    nex  =         3

     Term    38379    37680          -        0
     Intr    38854    38542          -        0
     Init    39115    38931          -        0

>4803878          /14132

    len  =     1582    nex  =         4

     Term    38379    37679          -        0
     Intr    38854    38542          -        0
     Intr    39082    38931          -        0
     Init    39260    39188          -        0

>4803878          /4936

    len  =      537    nex  =         1

     Sngl    45234    44698          -        0

>4803878          /2725

    len  =     1431    nex  =         4

     Term    51078    50648          -        0
     Intr    51328    51226          -        0
     Intr    51702    51580          -        0
     Init    52078    51914          -        0

>4803878          /10780

    len  =     1615    nex  =         3

     Init    52264    52570          +        0
     Intr    52981    53054          +        0
```

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Reference No. 2750-942P

```
    Term    53516   53878         +        0

>4803878        /40875

    len =     817    nex =        1

    Sngl    65721   66537         +        0

>4803878        /117183

    len =    1607    nex =        4

    Init    73114   73395         +        0
    Intr    73476   73685         +        0
    Intr    74219   74295         +        0
    Term    74422   74720         +        0

>4803878        /19202

    len =    1552    nex =        4

    Term    81492   81413         -        0
    Intr    81857   81669         -        0
    Intr    82005   81947         -        0
    Init    82964   82666         -        0

>4803878        /29968

    len =    1375    nex =        5

    Term    83510   83314         -        0
    Intr    83688   83599         -        0
    Intr    83874   83772         -        0
    Intr    84042   83962         -        0
    Init    84361   84132         -        0

>4803878        /21256

    len =    2506    nex =       10

    Init    88965   89156         +        0
    Intr    89749   89846         +        0
    Intr    89927   89969         +        0
    Intr    90054   90115         +        0
    Intr    90200   90256         +        0
    Intr    90345   90380         +        0
    Intr    90639   90711         +        0
    Intr    90777   90848         +        0
    Intr    90945   91048         +        0
    Term    91150   91470         +        0

>4803878        /30884

    len =    1120    nex =        5

    Term    91871   91785         -        0
    Intr    92048   91974         -        0
    Intr    92193   92137         -        0
    Intr    92413   92291         -        0
    Init    92898   92778         -        0
```

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```
>4803878        /6211

   len =     1416   nex =        5

    Term   91871    91565         -         0
    Intr   92048    91974         -         0
    Intr   92193    92137         -         0
    Intr   92413    92291         -         0
    Init   92980    92778         -         0

>4803878        /118823

   len =     1433   nex =        5

    Term   91871    91597         -         0
    Intr   92048    91974         -         0
    Intr   92193    92137         -         0
    Intr   92413    92291         -         0
    Init   93029    92778         -         0

>4803909        /33591

   len =     2548   nex =        7

    Init   25320    25450         +         0
    Intr   25545    25674         +         0
    Intr   25969    26075         +         0
    Intr   26572    26706         +         0
    Intr   26804    26907         +         0
    Intr   27030    27130         +         0
    Term   27226    27508         +         0

>4803909        /101918

   len =     2004   nex =        7

    Init   25331    25450         +         0
    Intr   25545    25674         +         0
    Intr   25969    26075         +         0
    Intr   26572    26706         +         0
    Intr   26804    26907         +         0
    Intr   27030    27130         +         0
    Term   27226    27334         +         0

>4803909        /35385

   len =     1648   nex =        2

    Init   34510    35420         +         0
    Term   35503    36157         +         0

>4803909        /36399

   len =     1999   nex =        5

    Term   74683    74273         -         0
    Intr   75005    74768         -         0
    Intr   75269    75203         -         0
    Intr   75560    75398         -         0
```

```
Init   76271   75939        -       0

>4803909        /14382

len =     572   nex =       1

Sngl   98753   98182        -       0

>4803919        /7141

len =     470   nex =       1

Sngl   10681   11150        +       0

>4803919        /20090

len =     638   nex =       3

Term   11473   11254        -       0
Intr   11717   11664        -       0
Init   11891   11797        -       0

>4803919        /19643

len =     515   nex =       1

Sngl   18105   18619        +       0

>4803919        /9942

len =     628   nex =       2

Term   18385   18182        -       0
Init   18466   18413        -       0

>4803919        /42149

len =     503   nex =       1

Sngl   18515   18013        -       0

>4803919        /37778

len =     570   nex =       1

Sngl   19660   20229        +       0

>4803919        /39351

len =    2416   nex =       9

Init   21856   22144        +       0
Intr   22554   22580        +       0
Intr   22672   22741        +       0
Intr   22863   22986        +       0
Intr   23146   23230        +       0
Intr   23321   23453        +       0
Intr   23544   23717        +       0
Intr   23785   23867        +       0
Term   23953   24271        +       0
```

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```
>4803919        /40766

    len =     2062    nex =        8

     Term    24902    24659          -          0
     Intr    25064    24993          -          0
     Intr    25345    25142          -          0
     Intr    25530    25453          -          0
     Intr    25707    25619          -          0
     Intr    25854    25804          -          0
     Intr    26112    25945          -          0
     Init   ·26720    26568          -          0

>4803919        /19342

    len =     2750    nex =       11

     Term    27185    26968          -          0
     Intr    27343    27269          -          0
     Intr    27502    27437          -          0
     Intr    27642    27589          -          0
     Intr    27860    27729          -          0
     Intr    28170    28094          -          0
     Intr    28344    28266          -          0
     Intr    28920    28805          -          0
     Intr    29072    29008          -          0
     Intr    29223    29158          -          0
     Init    29717    29489          -          0

>4803919        /35643

    len =      757    nex =        1

     Sngl    38824    39580          +          0

>4803919        /39721

    len =     2799    nex =        9

     Init    40435    40910          +          0
     Intr    41203    41259          +          0
     Intr    41359    41437          +          0
     Intr    41521    41681          +          0
     Intr    41788    41915          +          0
     Intr    42010    42147          +          0
     Intr    42474    42566          +          0
     Intr    42653    42773          +          0
     Term    42877    43233          +          0

>4803950        /7133

    len =     1106    nex =        2

     Term    15958    15293          -          0
     Init    16398    16136          -          0

>4809270        /21854

    len =     1810    nex =        6
```

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```
     Init   26325   26534        +        0
     Intr   26761   26841        +        0
     Intr   26913   27026        +        0
     Intr   27129   27407        +        0
     Intr   27488   27601        +        0
     Term   27698   27770        +        0

>4809270        /23558

    len =      674   nex =        1

     Sngl   40607   41280        +        0

>4809270        /36972

    len =     2847   nex =       11

     Init   64059   64119        +        0
     Intr   64238   64297        +        0
     Intr   64402   64477        +        0
     Intr   64578   64743        +        0
     Intr   64827   65082        +        0
     Intr   65192   65307        +        0
     Intr   65390   65610        +        0
     Intr   65694   65728        +        0
     Intr   65818   65917        +        0
     Intr   66016   66118        +        0
     Term   66195   66599        +        0

>4809270        /20668

    len =     1075   nex =        5

     Init   65522   65610        +        0
     Intr   65694   65728        +        0
     Intr   65818   65917        +        0
     Intr   66016   66118        +        0
     Term   66195   66596        +        0

>4809271        /21905

    len =     1099   nex =        2

     Term   12979   12614        -        0
     Init   13712   13449        -        0

>4809271        /22359

    len =     1095   nex =        1

     Sngl   13712   12618        -        0

>4809271        /848

    len =      760   nex =        1

     Sngl   33077   32318        -        0

>4809271        /102190
```

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```
      len =      743    nex =        1

        Sngl   33077   32335          -         0

>4809271        /21181

      len =      344    nex =        1

        Sngl    8636    8293          -         0

>4809271        /6687

      len =     1406    nex =        1

        Sngl    8731    7326          -         0

>4809294        /8411

      len =     1440    nex =        4

        Init   14573   14846          +         0
        Intr   15067   15169          +         0
        Intr   15295   15456          +         0
        Term   15734   16012          +         0

>4809294        /8986

      len =     2265    nex =        7

        Init   21367   21498          +         0
        Intr   21594   21784          +         0
        Intr   21897   22034          +         0
        Intr   22122   22258          +         0
        Intr   22349   22514          +         0
        Intr   22682   22921          +         0
        Term   23003   23631          +         0

>4809294        /33512

      len =     1150    nex =        2

        Term   30857   30449          -         0
        Init   31590   31115          -         0

>4809295        /3676

      len =     2083    nex =        6

        Term   53797   53671          -         0
        Intr   53990   53913          -         0
        Intr   54177   54096          -         0
        Intr   54877   54684          -         0
        Intr   55272   55181          -         0
        Init   55753   55348          -         0

>4809296        /30187

      len =     1390    nex =        3
```

```
     Term   20449   20131        -        0
     Intr   20937   20890        -        0
     Init   21511   21158        -        0

>4809296        /3071

    len =     1092    nex =       4

     Init     411     493        +        0
     Intr     704     768        +        0
     Intr     971    1041        +        0
     Term    1133    1359        +        0

>4809296        /34078

    len =     1150    nex =       4

     Init     411     493        +        0
     Intr     704     768        +        0
     Intr     971    1041        +        0
     Term    1133    1416        +        0

>4835223        /36702

    len =     2050    nex =       8

     Term   28902   28573        -        0
     Intr   29030   28977        -        0
     Intr   29207   29113        -        0
     Intr   29404   29307        -        0
     Intr   29625   29483        -        0
     Intr   29839   29708        -        0
     Intr   30116   29988        -        0
     Init   30618   30194        -        0

>4835223        /3286

    len =      562    nex =       1

     Sngl   40069   39514        -        0

>4835223        /8864

    len =     1585    nex =       4

     Term   42999   42518        -        0
     Intr   43254   43184        -        0
     Intr   43459   43352        -        0
     Init   44102   43814        -        0

>4835223        /153949

    len =     1900    nex =       6

     Init   50935   51051        +        0
     Intr   51154   51196        +        0
     Intr   51320   51381        +        0
     Intr   51479   51575        +        0
     Intr   52119   52189        +        0
     Term   52348   52439        +        0
```

```
>4835223        /27213

   len =    1570   nex =       2

    Init   59627   60267        +        0
    Term   60638   61187        +        0

>4835223        /732

   len =    1474   nex =       1

    Sngl   62121   62953        +        0

>4835223        /20695

   len =    1163   nex =       0

>4835223        /23377

   len =     790   nex =       1

    Sngl   65775   64987        -        0

>4835223        /10044

   len =     495   nex =       1

    Sngl   66699   67193        +        0

>4835223        /32364

   len =    1750   nex =       1

    Sngl   78553   80295        +        0

>4835223        /5193

   len =    1348   nex =       8

    Term   87820   87718        -        0
    Intr   87968   87907        -        0
    Intr   88132   88060        -        0
    Intr   88361   88219        -        0
    Intr   88494   88447        -        0
    Intr   88643   88569        -        0
    Intr   88863   88780        -        0
    Init   89065   88950        -        0

>4835773        /1989

   len =     690   nex =       1

    Sngl   20133   20822        +        0

>4835773        /34385

   len =    2830   nex =      12

    Init    2024    2254        +        0
```

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```
     Intr    2371    2450          +         0
     Intr    2617    2733          +         0
     Intr    2833    2936          +         0
     Intr    3023    3089          +         0
     Intr    3363    3496          +         0
     Intr    3582    3630          +         0
     Intr    3767    3838          +         0
     Intr    3929    4030          +         0
     Intr    4116    4183          +         0
     Intr    4369    4521          +         0
     Term    4602    4852          +         0

>4835773        /6281

    len =    1946   nex =        7

     Init   42427   42503          +         0
     Intr   42604   42707          +         0
     Intr   42803   42903          +         0
     Intr   42991   43094          +         0
     Intr   43278   43715          +         0
     Intr   43797   43928          +         0
     Term   43997   44372          +         0

>4835773        /28214

    len =    1757   nex =        3

     Term   48583   47836          -         0
     Intr   48864   48677          -         0
     Init   49592   48965          -         0

>4835773        /21943

    len =    1870   nex =        4

     Init    5082    5505          +         0
     Intr    5583    5729          +         0
     Intr    5834    6043          +         0
     Term    6473    6945          +         0

>4836442        /1137

    len =     730   nex =        1

     Sngl    5764    6489          +         0

>4836906        /93312

    len =    1831   nex =        4

     Term   26269   26014          -         0
     Intr   26453   26358          -         0
     Intr   26664   26531          -         0
     Init   27844   26730          -         0

>4836906        /29867

    len =     831   nex =        1
    Sngl   64559   63729         -        0

>4836906       /14139

   len =     1570   nex =        2

    Term   80467   80066         -        0
    Init   80887   80566         -        0

>4836906       /5665

   len =      898   nex =        2

    Init   86401   86906         +        0
    Term   87082   87298         +        0

>4850281       /4143

   len =      436   nex =        1

    Sngl  107876  108311         +        0

>4850409       /12642

   len =      583   nex =        2

    Term  104024  103684         -        0
    Init  104266  104150         -        0

>4850409       /20585

   len =      730   nex =        2

    Init  106425  106547         +        0
    Term  106829  107153         +        0

>4850409       /28536

   len =      296   nex =        1

    Sngl  106829  107124         +        0

>4850409       /39306

   len =     2314   nex =        5

    Init   14095   14194         +        0
    Intr   14334   14483         +        0
    Intr   14590   14863         +        0
    Intr   14895   14982         +        0
    Term   15124   15585         +        0

>4850409       /3062

   len =     2530   nex =        7

    Term   18950   18748         -        0
    Intr   19135   19019         -        0
    Intr   19377   19225         -        0
    Intr   19637   19467         -        0
```

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```
     Intr    19900    19721        -        0
     Intr    20082    19984        -        0
     Init    20494    20407        -        0

>4850409         /4791

    len =      736    nex =        0

>4850409         /27686

    len =      716    nex =        1

     Sngl    23412    24127        +        0

>4850409         /30722

    len =     1677    nex =        4

     Init    34646    34737        +        0
     Intr    34898    35330        +        0
     Intr    35419    35472        +        0
     Term    35528    35913        +        0

>4850409         /94765

    len =      235    nex =        1

     Sngl    35586    35820        +        0

>4850409         /40551

    len =     1848    nex =        4

     Term    62292    61937        -        0
     Intr    62986    62451        -        0
     Intr    63104    63016        -        0
     Init    63784    63510        -        0

>4850409         /112273

    len =      850    nex =        2

     Term    66692    66564        -        0
     Init    66880    66784        -        0

>4850409         /5724

    len =     1295    nex =        2

     Term    66692    66532        -        0
     Init    66880    66784        -        0

>4850409         /2548

    len =      692    nex =        1

     Sngl    67705    68396        +        0

>4850409         /8711
```

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```
   len =      708    nex =        1

    Sngl   67705    68412          +        0

>4850409        /2535

   len =      598    nex =        1

    Sngl   69019    69616          +        0

>4850409        /30316

   len =      790    nex =        1

    Sngl   76323    75925          -        0

>4850409        /7539

   len =      791    nex =        1

    Sngl   76452    75925          -        0

>4850409        /4289

   len =     1643    nex =        1

    Sngl   90019    91371          +        0

>4850409        /27754

   len =     1431    nex =        1

    Sngl   89946    91376          +        0

>4850411        /21257

   len =      430    nex =        1

    Sngl   14482    14057          -        0

>4874280        /12402

   len =     1492    nex =        4

    Init  100913   101010          +        0
    Intr  101624   101737          +        0
    Intr  101836   101915          +        0
    Term  102002   102400          +        0

>4874280        /13543

   len =      670    nex =        1

    Sngl  110726   110057          -        0

>4874280        /29810

   len =     1717    nex =        7

    Term  115803   115457          -        0
    Intr  116038  115976        -       0
    Intr  116268  116158        -       0
    Intr  116431  116349        -       0
    Intr  116759  116512        -       0
    Intr  116980  116882        -       0
    Init  117173  117074        -       0

>4874280        /14469

    len =     146    nex =        1

    Sngl   25949   25804        -       0

>4874280        /1871

    len =    1210    nex =        1

    Sngl   37446   36737        -       0

>4874280        /25201

    len =    1044    nex =        6

    Term   43874   43821        -       0
    Intr   44112   43996        -       0
    Intr   44365   44198        -       0
    Intr   44512   44447        -       0
    Intr   44684   44589        -       0
    Init   44864   44764        -       0

>4874280        /10068

    len =     740    nex =        1

    Sngl   57061   56322        -       0

>4874280        /11912

    len =     772    nex =        4

    Term   62694   62514        -       0
    Intr   62885   62784        -       0
    Intr   63127   62992        -       0
    Init   63285   63202        -       0

>4874280        /30239

    len =     758    nex =        1

    Sngl   63635   64392        +       0

>4874280        /22166

    len =    1400    nex =        4

    Term   64736   64526        -       0
    Intr   65314   65273        -       0
    Intr   65723   65668        -       0
    Init   65925   65828        -       0
```

```
>4874280        /8886

   len =      271    nex =       1

    Sngl    83252    82982        -        0

>4874280        /21847

   len =     1253    nex =       2

    Init    88169    88958        +        0
    Term    89032    89421        +        0

>4874280        /19135

   len =     5291    nex =       3

    Term    86468    86081        -        0
    Intr    87158    86630        -        0
    Init    91371    91235        -        0

>4874280        /16209

   len =     1870    nex =       2

    Term    90809    89582        -        0
    Init    91445    91235        -        0

>4874280        /9177

   len =     1603    nex =       5

    Term    92357    92087        -        0
    Intr    92498    92429        -        0
    Intr    92677    92587        -        0
    Intr    93017    92768        -        0
    Init    93689    93526        -        0

>4874280        /17356

   len =     1051    nex =       1

    Sngl    97810    98457        +        0

>4874280        /121746

   len =      752    nex =       1

    Sngl    97770    98521        +        0

>4874281        /18634

   len =      992    nex =       1

    Sngl    28766    29757        +        0

>4878038        /33093

   len =     1838    nex =       4
```

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```
     Init   17045   17083        +        0
     Intr   17496   17715        +        0
     Intr   17935   18104        +        0
     Term   18283   18716        +        0

>4878038        /12279

    len =     1657   nex =        4

     Init   17042   17083        +        0
     Intr   17496   17715        +        0
     Intr   17935   18104        +        0
     Term   18283   18698        +        0

>4878038        /41419

    len =      101   nex =        1

     Sngl    1922    1822        -        0

>4878038        /103167

    len =      827   nex =        3

     Term   22122   21766        -        0
     Intr   22412   22340        -        0
     Init   22592   22509        -        0

>4878038        /12044

    len =      832   nex =        3

     Term   22122   21767        -        0
     Intr   22412   22340        -        0
     Init   22598   22509        -        0

>4878038        /7414

    len =      850   nex =        3

     Term   22122   21767        -        0
     Intr   22412   22340        -        0
     Init   22611   22509        -        0

>4878038        /39977

    len =      915   nex =        3

     Term   22122   21728        -        0
     Intr   22412   22340        -        0
     Init   22642   22509        -        0

>4878038        /18748

    len =      635   nex =        3

     Term   22122   22008        -        0
     Intr   22412   22340        -        0
     Init   22642   22509        -        0
```

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```
>4878038        /34623

   len =      911    nex =        3

    Term   22122    21766          -          0
    Intr   22412    22340          -          0
    Init   22676    22509          -          0

>4878038        /35553

   len =     1034    nex =        3

    Init   23065    23187          +          0
    Intr   23268    23447          +          0
    Term   23838    24098          +          0

>4878038        /40238

   len =     1210    nex =        1

    Sngl    2380     1177          -          0

>4878038        /107233

   len =      643    nex =        4

    Term   48867    48732          -          0
    Intr   49015    48983          -          0
    Intr   49222    49136          -          0
    Init   49374    49316          -          0

>4878038        /35098

   len =     2170    nex =        3

    Init   53312    53618          +          0
    Intr   53769    54355          +          0
    Term   55015    55476          +          0

>4878038        /6413

   len =     3951    nex =        5

    Init   56661    56949          +          0
    Intr   58951    59082          +          0
    Intr   59203    59289          +          0
    Intr   59403    59453          +          0
    Term   59530    60611          +          0

>4878038        /119807

   len =     1461    nex =        3

    Init   59122    59289          +          0
    Intr   59403    59453          +          0
    Term   60354    60582          +          0

>4878038        /34128

   len =     1012    nex =        3
```

```
      Init   65143   65373        +      0
      Intr   65735   65837        +      0
      Term   66104   66154        +      0

>4878038        /123625

    len =      897    nex =       2

      Init   69908   70156        +      0
      Term   70242   70804        +      0

>4878038        /150460

    len =      313    nex =       1

      Sngl   75227   74915        -      0

>4878039        /27589

    len =     1529    nex =       2

      Init   21799   21871        +      0
      Term   22351   22674        +      0

>4878039        /31701

    len =     1491    nex =       2

      Init   21799   21871        +      0
      Term   22351   22658        +      0

>4878039        /22285

    len =     1224    nex =       1

      Sngl   23812   23609        -      0

>4878039        /7311

    len =     1244    nex =       1

      Sngl   23835   23609        -      0

>4878039        /13410

    len =     1243    nex =       0

>4878039        /11384

    len =      534    nex =       1

      Sngl   41117   40584        -      0

>4878039        /21735

    len =     2391    nex =       6

      Init    6076    6252        +      0
      Intr    6656    6778        +      0
```

```
     Intr    6934    7050        +       0
     Intr    7141    7336        +       0
     Intr    7584    7698        +       0
     Term    7795    7959        +       0

>4878039        /153670

    len =    2383    nex =       6

     Init    6084    6252        +       0
     Intr    6656    6778        +       0
     Intr    6934    7050        +       0
     Intr    7141    7336        +       0
     Intr    7584    7698        +       0
     Term    7795    7959        +       0

>4878039        /118900

    len =    2308    nex =       6

     Init    6091    6252        +       0
     Intr    6656    6778        +       0
     Intr    6934    7050        +       0
     Intr    7141    7336        +       0
     Intr    7584    7698        +       0
     Term    7795    7959        +       0

>4878039        /17026

    len =     156    nex =       1

     Sngl   61886   61731        -       0

>4878039        /24663

    len =     334    nex =       1

     Sngl   61970   61637        -       0

>4878039        /15897

    len =     458    nex =       1

     Sngl   62053   61601        -       0

>4878039        /9325

    len =    1366    nex =       4

     Term   62075   61726        -       0
     Intr   62381   62331        -       0
     Intr   62704   62472        -       0
     Init   63091   62815        -       0

>4878039        /33019

    len =    1400    nex =       4

     Term   62075   61700        -       0
     Intr   62381   62331        -       0
     Intr    62704    62472         -         0
     Init    63099    62815         -         0

>4878039        /14650

    len =      190    nex =        1

     Sngl    63116    62933         -         0

>4878039        /41423

    len =     1419    nex =        0

>4878039        /33922

    len =     1522    nex =        4

     Term    62075    61601         -         0
     Intr    62381    62331         -         0
     Intr    62704    62472         -         0
     Init    63122    62815         -         0

>4878039        /8579

    len =      813    nex =        3

     Init    72885    73192         +         0
     Intr    73271    73362         +         0
     Term    73447    73688         +         0

>4878039        /38014

    len =     1795    nex =        3

     Term    81714    81447         -         0
     Intr    81951    81802         -         0
     Init    82973    82025         -         0

>4878039        /6888

    len =     1128    nex =        2

     Term    84567    84273         -         0
     Init    85400    84860         -         0

>4883587        /206485

    len =      538    nex =        1

     Sngl   113867   113330         -         0

>4883587        /8904

    len =     1657    nex =        4

     Init     1398     1648         +         0
     Intr     2403     2594         +         0
     Intr     2697     2865         +         0
     Term     2973     3054         +         0
```

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```
>4883587       /99140

   len =    612   nex =      1

    Sngl  81836   81225        -        0

>4883587       /35520

   len =   1890   nex =      1

    Sngl  83089   81200        -        0

>4883588       /30158

   len =   1058   nex =      3

    Init  78085   78219        +        0
    Intr  78340   78388        +        0
    Term  78702   79142        +        0

>4883588       /42159

   len =    938   nex =      3

    Init  78151   78219        +        0
    Intr  78340   78388        +        0
    Term  78702   79088        +        0

>4883588       /117103

   len =    956   nex =      2

    Init  78187   78219        +        0
    Term  78340   79142        +        0

>4883588       /12963

   len =   2950   nex =     10

    Term  79984   79379        -        0
    Intr  80178   80092        -        0
    Intr  80331   80272        -        0
    Intr  80484   80416        -        0
    Intr  80729   80573        -        0
    Intr  80924   80848        -        0
    Intr  81328   81283        -        0
    Intr  81508   81422        -        0
    Intr  81730   81584        -        0
    Init  82321   81955        -        0

>4883589       /39471

   len =    910   nex =      1

    Sngl  27167   26259        -        0

>4883595       /27178

   len =   1305   nex =      5
```

```
     Init    13666    13838          +         0
     Intr    14082    14123          +         0
     Intr    14233    14403          +         0
     Intr    14555    14641          +         0
     Term    14739    14970          +         0

>4883595        /28643

    len =     1274    nex =        5

     Init    13690    13838          +         0
     Intr    14082    14123          +         0
     Intr    14233    14403          +         0
     Intr    14555    14641          +         0
     Term    14739    14963          +         0

>4883595        /27627

    len =     1278    nex =        5

     Init    13690    13838          +         0
     Intr    14082    14123          +         0
     Intr    14233    14403          +         0
     Intr    14555    14641          +         0
     Term    14739    14967          +         0

>4883595        /121982

    len =     1275    nex =        5

     Init    13695    13838          +         0
     Intr    14082    14123          +         0
     Intr    14233    14403          +         0
     Intr    14555    14641          +         0
     Term    14739    14969          +         0

>4883595        /25350

    len =     1004    nex =        5

     Init    13767    13838          +         0
     Intr    14082    14123          +         0
     Intr    14233    14403          +         0
     Intr    14555    14641          +         0
     Term    14739    14764          +         0

>4883595        /32137

    len =     1896    nex =        5

     Init    38588    38819          +         0
     Intr    39455    39511          +         0
     Intr    39688    39878          +         0
     Intr    39967    40071          +         0
     Term    40162    40483          +         0

>4883595        /37242

    len =     1971    nex =        5
```

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```
     Init   38588   38819        +       0
     Intr   39455   39511        +       0
     Intr   39688   39878        +       0
     Intr   39967   40071        +       0
     Term   40162   40558        +       0

>4883595      /20760

    len =    1990   nex =       5

     Init   38780   38819        +       0
     Intr   39455   39511        +       0
     Intr   39688   39878        +       0
     Intr   39967   40071        +       0
     Term   40162   40576        +       0

>4883595      /34869

    len =     899   nex =       1

     Sngl   38590   38819        +       0

>4883595      /25104

    len =     372   nex =       1

     Sngl   40223   40578        +       0

>4883595      /5109

    len =    1376   nex =       4

     Term   66464   66136        -       0
     Intr   66734   66627        -       0
     Intr   66906   66815        -       0
     Init   67219   67018        -       0

>4883595      /113853

    len =     912   nex =       3

     Init   67833   68023        +       0
     Intr   68119   68397        +       0
     Term   68518   68744        +       0

>4883595      /27387

    len =    1095   nex =       1

     Sngl   97707   96779        -       0

>4883598      /112395

    len =     102   nex =       1

     Sngl   22321   22422        +       0

>4883598      /27175

    len =    1292   nex =       6
    Term   53471   53305          -         0
    Intr   53639   53577          -         0
    Intr   53815   53735          -         0
    Intr   53963   53919          -         0
    Intr   54304   54215          -         0
    Init   54443   54387          -         0

>4883598        /31249

   len =     1066    nex =        3

    Init   75625   75768          +         0
    Intr   75862   75941          +         0
    Term   76089   76422          +         0

>4883599        /41933

   len =     2470    nex =        6

    Init  107705  108086          +         0
    Intr  108321  108417          +         0
    Intr  108494  108609          +         0
    Intr  109315  109359          +         0
    Intr  109639  109774          +         0
    Term  109859  110173          +         0

>4883599        /19094

   len =     1768    nex =        1

    Sngl  118037  116270          -         0

>4883599        /11488

   len =      696    nex =        1

    Sngl  119284  118589          -         0

>4883599        /97088

   len =     1795    nex =        4

    Term   11068   10803          -         0
    Intr   11765   11697          -         0
    Intr   12260   12150          -         0
    Init   12597   12343          -         0

>4883599        /7831

   len =     2010    nex =        4

    Term   34414   33824          -         0
    Intr   35311   35094          -         0
    Intr   35494   35394          -         0
    Init   35833   35596          -         0

>4883599        /13320

   len =     2015    nex =        1
```

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```
  Sngl    42074   40567         -         0

>4883599        /29157

  len =     1707   nex =        6

  Term    43450   43184         -         0
  Intr    43605   43533         -         0
  Intr    43874   43750         -         0
  Intr    44036   43965         -         0
  Intr    44322   44225         -         0
  Init    44890   44558         -         0

>4883599        /16221

  len =     1270   nex =        5

  Term    50525   50232         -         0
  Intr    50711   50610         -         0
  Intr    50909   50793         -         0
  Intr    51085   50985         -         0
  Init    51498   51268         -         0

>4883599        /2416

  len =     1352   nex =        4

  Term    52955   52650         -         0
  Intr    53295   53025         -         0
  Intr    53806   53522         -         0
  Init    54001   53894         -         0

>4883599        /36633

  len =     1133   nex =        2

  Init     6605    7052         +         0
  Term     7144    7737         +         0

>4883599        /119288

  len =      646   nex =        2

  Init     6611    7052         +         0
  Term     7144    7256         +         0

>4883599        /12558

  len =     2034   nex =        3

  Init    66457   66727         +         0
  Intr    66744   66880         +         0
  Term    66958   68472         +         0

>4883599        /12259

  len =      212   nex =        1

  Sngl    67175   67386         +         0
```

```
>4883599        /39855

   len =     1599    nex =        4

     Term   71283    71095        -         0
     Intr   71609    71547        -         0
     Intr   71898    71722        -         0
     Init   72208    72146        -         0

>4883599        /19570

   len =     1714    nex =        3

     Init   76320    76853        +         0
     Intr   77103    77181        +         0
     Term   77311    78033        +         0

>4883599        /3766

   len =      631    nex =        2

     Term   98968    98595        -         0
     Init   99225    99034        -         0

>4884020        /8077

   len =     1751    nex =        3

     Term   15439    14937        -         0
     Intr   15776    15562        -         0
     Init   16497    16374        -         0

>4884020        /5161

   len =     2037    nex =        8

     Term   22090    22010        -         0
     Intr   22315    22187        -         0
     Intr   22607    22491        -         0
     Intr   22782    22718        -         0
     Intr   22906    22867        -         0
     Intr   23079    23017        -         0
     Intr   23444    23183        -         0
     Init   23627    23517        -         0

>4884020        /27539

   len =     1335    nex =        4

     Init   34674    35251        +         0
     Intr   35340    35450        +         0
     Intr   35592    35705        +         0
     Term   35914    36008        +         0

>4884021        /2419

   len =     1136    nex =        2

     Init   33897    34058        +         0
```

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```
    Term   34433   35032        +        0

>4884021        /19314

    len =    2613   nex =        2

    Init    3435    3738        +        0
    Term    3868    6047        +        0

>4884022        /2982

    len =    2050   nex =        8

    Init   25501   25640        +        0
    Intr   25886   26005        +        0
    Intr   26146   26292        +        0
    Intr   26381   26476        +        0
    Intr   26560   26655        +        0
    Intr   26927   27044        +        0
    Intr   27132   27204        +        0
    Term   27301   27548        +        0

>4884022        /40445

    len =    3512   nex =       12

    Term   28180   27923        -        0
    Intr   28424   28270        -        0
    Intr   28550   28502        -        0
    Intr   28909   28653        -        0
    Intr   29159   29030        -        0
    Intr   29337   29269        -        0
    Intr   29632   29579        -        0
    Intr   29897   29739        -        0
    Intr   30278   30141        -        0
    Intr   30434   30372        -        0
    Intr   30577   30538        -        0
    Init   30707   30661        -        0

>4884023        /94438

    len =    1247   nex =        3

    Init   32053   32253        +        0
    Intr   32590   32658        +        0
    Term   32956   33299        +        0

>4884023        /40485

    len =    2060   nex =        7

    Init   76282   76657        +        0
    Intr   76812   76874        +        0
    Intr   77011   77127        +        0
    Intr   77202   77448        +        0
    Intr   77547   77705        +        0
    Intr   77780   77931        +        0
    Term   78015   78341        +        0

>4884023        /110908
```

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```
    len =      372    nex =        0

 >4886265         /16125

    len =     1665    nex =        4

      Init   15167    15326         +         0
      Intr   15839    15978         +         0
      Intr   16219    16344         +         0
      Term   16429    16831         +         0

 >4886265         /39625

    len =      372    nex =        1

      Sngl   45373    45744         +         0

 >4886265         /11782

    len =      910    nex =        1

      Sngl   58085    58990         +         0

 >4886265         /92178

    len =      910    nex =        1

      Sngl   60596    59692         -         0

 >4886265         /7969

    len =     1105    nex =        5

      Term    6613     6546         -         0
      Intr    6828     6699         -         0
      Intr    7010     6925         -         0
      Intr    7452     7407         -         0
      Init    7643     7530         -         0

 >4886265         /9716

    len =     1646    nex =        6

      Term    6613     6377         -         0
      Intr    6828     6699         -         0
      Intr    7010     6925         -         0
      Intr    7452     7407         -         0
      Intr    7649     7530         -         0
      Init    8022     7921         -         0

 >4886265         /37248

    len =     1450    nex =        2

      Init   84108    84785         +         0
      Term   84958    85556         +         0

 >4886265         /14246
```

09595326.061600

```
   len =    1696   nex =       4

    Term  88532   88155       -         0
    Intr  88912   88604       -         0
    Intr  89088   88987       -         0
    Init  89850   89191       -         0

>4887257        /145295

   len =    1695   nex =       4

    Term  10323   10083       -         0
    Intr  10562   10518       -         0
    Intr  10742   10642       -         0
    Init  11039   10834       -         0

>4887257        /22508

   len =    1006   nex =       4

    Term  23643   23309       -         0
    Intr  23922   23824       -         0
    Intr  24104   24023       -         0
    Init  24314   24189       -         0

>4887257        /143886

   len =     850   nex =       3

    Init  69630   69796       +         0
    Intr  69886   69951       +         0
    Term  70176   70475       +         0

>4887257        /39490

   len =    2950   nex =      12

    Term  74958   74703       -         0
    Intr  75114   75047       -         0
    Intr  75239   75176       -         0
    Intr  75402   75321       -         0
    Intr  75574   75516       -         0
    Intr  75705   75646       -         0
    Intr  75982   75820       -         0
    Intr  76205   76081       -         0
    Intr  76563   76501       -         0
    Intr  76761   76702       -         0
    Intr  77059   76880       -         0
    Init  77646   77488       -         0

>4887257        /8633

   len =    2801   nex =      10

    Init  78250   78326       +         0
    Intr  78410   78474       +         0
    Intr  78598   78644       +         0
    Intr  78737   78812       +         0
    Intr  78900   78967       +         0
    Intr  79275   79344       +         0
```

09555525.051500

```
     Intr    79628    79713         +         0
     Intr    79880    79942         +         0
     Intr    80237    80302         +         0
     Term    80400    80609         +         0

>4887257        /109912

    len =     1124    nex =        3

     Init    94040    94098         +         0
     Intr    94128    94314         +         0
     Term    94452    95163         +         0

>4887737        /2004

    len =      675    nex =        1

     Sngl     7907     8581         +         0

>4887737        /1505

    len =      673    nex =        1

     Sngl     7909     8581         +         0

>4887737        /31675

    len =      599    nex =        1

     Sngl     7913     8511         +         0

>4887737        /106951

    len =      677    nex =        1

     Sngl     7913     8589         +         0

>4887737        /108174

    len =     1630    nex =        2

     Term    85528    84876         -         0
     Init    86500    86083         -         0

>4887737        /33557

    len =     1788    nex =        2

     Term    85528    84753         -         0
     Init    86540    86083         -         0

>4887738        /23872

    len =      764    nex =        1

     Sngl    20581    19818         -         0

>4887738        /119765

    len =     3139    nex =        7
```

09595329.061600

```
        Init    3396    4329        +       0
        Intr    4533    4587        +       0
        Intr    5001    5039        +       0
        Intr    5122    5185        +       0
        Intr    5549    5576        +       0
        Intr    5741    5832        +       0
        Term    6013    6534        +       0

  >4887738        /114822

       len =     3136    nex =       7

        Init    3399    4329        +       0
        Intr    4533    4587        +       0
        Intr    5001    5039        +       0
        Intr    5122    5185        +       0
        Intr    5549    5576        +       0
        Intr    5741    5832        +       0
        Term    6013    6534        +       0

  >4887738        /28177

       len =     1570    nex =       3

        Term    43839   43444       -       0
        Intr    44038   43918       -       0
        Init    45006   44725       -       0

  >4887738        /6612

       len =     2489    nex =       4

        Init    61698   61850       +       0
        Intr    61949   62099       +       0
        Intr    62198   62262       +       0
        Term    62746   63244       +       0

  >4887738        /29992

       len =      926    nex =       2

        Term    78325   77774       -       0
        Init    78699   78401       -       0

  >4887738        /146828

       len =      507    nex =       1

        Sngl    83673   83167       -       0

  >4887740        /33192

       len =     2290    nex =       5

        Init      199     824       +       0
        Intr      922    1089       +       0
        Intr     1185    1376       +       0
        Intr     1551    1619       +       0
        Term     1985    2290       +       0
```

```
>4887740        /23467

   len =      521    nex =        2

    Init   46831    46988          +          0
    Term   47080    47203          +          0

>4887740        /12467

   len =      651    nex =        1

    Sngl    5943     5293          -          0

>4895147        /124537

   len =     1961    nex =        7

    Init   15249    15368          +          0
    Intr   15727    15850          +          0
    Intr   15938    15966          +          0
    Intr   16050    16115          +          0
    Intr   16202    16309          +          0
    Intr   16399    16464          +          0
    Term   16795    16876          +          0

>4895147        /21739

   len =     1469    nex =        4

    Term   50700    50490          -          0
    Intr   51095    50898          -          0
    Intr   51286    51185          -          0
    Init   51958    51514          -          0

>4895147        /21920

   len =      444    nex =        1

    Sngl   53076    52638          -          0

>4895147        /37658

   len =     1662    nex =        3

    Term   53123    52698          -          0
    Intr   53993    53216          -          0
    Init   54359    54077          -          0

>4895147        /24367

   len =      993    nex =        3

    Term   55478    55257          -          0
    Intr   55772    55560          -          0
    Init   56104    56024          -          0

>4895147        /20633

   len =     1066    nex =        3
```

```
     Term   55478   55258          -         0
     Intr   55772   55560          -         0
     Init   56104   56024          -         0

>4895147        /39533

   len =     730    nex =        2

     Term   55772   55601          -         0
     Init   56104   56024          -         0

>4895147        /4458

   len =     740    nex =        2

     Term   55772   55598          -         0
     Init   56104   56024          -         0

>4895147        /20676

   len =    1041    nex =        2

     Term   55478   55286          -         0
     Init   56104   56024          -         0

>4895147        /524

   len =    1101    nex =        1

     Sngl   56104   56024          -         0

>4895147        /6803

   len =     979    nex =        3

     Term   55478   55354          -         0
     Intr   55772   55560          -         0
     Init   56104   56024          -         0

>4895147        /15992

   len =    1018    nex =        3

     Term   55478   55318          -         0
     Intr   55772   55560          -         0
     Init   56104   56024          -         0

>4895147        /24607

   len =     550    nex =        1

     Sngl   68923   68382          -         0

>4895147        /43068

   len =    2470    nex =       10

     Term   70358   70271          -         0
     Intr   70597   70472          -         0
```

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```
    Intr    70793    70704            -          0
    Intr    71005    70880            -          0
    Intr    71301    71248            -          0
    Intr    71483    71403            -          0
    Intr    71698    71591            -          0
    Intr    71890    71819            -          0
    Intr    72038    71976            -          0
    Init    72731    72465            -          0

>4895147        /20749

   len =     2192    nex =        6

    Init     7453     7722            +          0
    Intr     7862     7924            +          0
    Intr     8068     8184            +          0
    Intr     8583     8988            +          0
    Intr     9110     9261            +          0
    Term     9343     9644            +          0

>4895147        /28975

   len =     1253    nex =        4

    Init    77491    77810            +          0
    Intr    78228    78304            +          0
    Intr    78428    78536            +          0
    Term    78629    78743            +          0

>4895147        /98397

   len =      730    nex =        1

    Sngl    79391    79138            -          0

>4895147        /37526

   len =     1066    nex =        1

    Sngl    80755    81820            +          0

>4895148        /14553

   len =     1704    nex =        2

    Term    39478    38899            -          0
    Init    40602    39746            -          0

>4895148        /114523

   len =      310    nex =        1

    Sngl    55690    55388            -          0

>4895148        /40329

   len =     1066    nex =        2

    Term    55806    55404            -          0
    Init    56469    56070            -          0
```

09856356.051502

```
>4895148        /109156

    len =      480    nex =       1

     Sngl   57879   57400         -        0

>4895149        /37283

    len =     1572    nex =       2

     Term   20933   20082         -        0
     Init   21653   21105         -        0

>4895167        /18533

    len =      892    nex =       3

     Init   43380   43470         +        0
     Intr   43555   43730         +        0
     Term   43825   44256         +        0

>4895176        /34547

    len =     2128    nex =       6

     Term  104785  104678         -        0
     Intr  105146  104871         -        0
     Intr  105483  105396         -        0
     Intr  105659  105583         -        0
     Intr  106058  106008         -        0
     Init  106328  106140         -        0

>4895176        /6630

    len =     1966    nex =       6

     Term  111441  111255         -        0
     Intr  112230  111955         -        0
     Intr  112394  112307         -        0
     Intr  112556  112480         -        0
     Intr  112906  112856         -        0
     Init  113220  112995         -        0

>4895176        /7663

    len =     2007    nex =       5

     Term  111441  111237         -        0
     Intr  112230  111955         -        0
     Intr  112394  112307         -        0
     Intr  112556  112480         -        0
     Init  112906  112856         -        0

>4895176        /40538

    len =     2556    nex =       8

     Term   42176   41886         -        0
     Intr   42395   42282         -        0
     Intr   42663   42480        -         0
     Intr   42988   42871        -         0
     Intr   43261   43133        -         0
     Intr   43455   43404        -         0
     Intr   43792   43642        -         0
     Init   44441   44284        -         0

>4895176        /109069

     len =    1787   nex =       2

     Init   51902   51959        +         0
     Term   52945   53244        +         0

>4895176        /18810

     len =     829   nex =       1

     Sngl   54124   53296        -         0

>4895176        /11827

     len =     862   nex =       1

     Sngl   54143   53282        -         0

>4895176        /2445

     len =    2175   nex =      11

     Term   65959   65856        -         0
     Intr   66105   66059        -         0
     Intr   66298   66203        -         0
     Intr   66481   66382        -         0
     Intr   66642   66582        -         0
     Intr   66796   66725        -         0
     Intr   66979   66899        -         0
     Intr   67227   67159        -         0
     Intr   67424   67338        -         0
     Intr   67812   67723        -         0
     Init   68030   67896        -         0

>4895176        /912

     len =    2890   nex =      12

     Term   65760   65465        -         0
     Intr   65959   65853        -         0
     Intr   66105   66059        -         0
     Intr   66298   66203        -         0
     Intr   66481   66382        -         0
     Intr   66642   66582        -         0
     Intr   66796   66725        -         0
     Intr   66979   66899        -         0
     Intr   67227   67159        -         0
     Intr   67424   67338        -         0
     Intr   67812   67723        -         0
     Init   68345   67896        -         0

>4895176        /10987
```

```
len =      914    nex =        1

 Sngl   75026   75939         +         0

>4895176         /34976

len =      986    nex =        1

 Sngl   84464   83479         -         0

>4895213         /17709

len =      215    nex =        1

 Sngl   12033   11819         -         0

>4895213         /19452

len =     1181    nex =        4

  Term   12235   11861         -         0
  Intr   12448   12322         -         0
  Intr   12634   12559         -         0
  Init   13041   12797         -         0

>4895213         /168

len =     1128    nex =        2

  Term   21760   21460         -         0
  Init   22587   22368         -         0

>4895213         /35168

len =     2411    nex =        6

  Init   69033   69478         +         0
  Intr   69674   69795         +         0
  Intr   69886   69976         +         0
  Intr   70067   70187         +         0
  Intr   70283   70356         +         0
  Term   70828   71443         +         0

>4895233         /19714

len =     1933    nex =        7

  Term   14019   13908         -         0
  Intr   14335   14258         -         0
  Intr   14918   14868         -         0
  Intr   15157   14999         -         0
  Intr   15354   15239         -         0
  Intr   15541   15460         -         0
  Init   15691   15621         -         0

>4895233         /6457

len =     2356    nex =        8
```

```
Term   14019   13655       -        0
Intr   14335   14258       -        0
Intr   14918   14868       -        0
Intr   15157   14999       -        0
Intr   15354   15239       -        0
Intr   15541   15460       -        0
Intr   15691   15621       -        0
Init   16010   15800       -        0

>4895233        /19091

  len =    1316   nex =       0

>4895233        /2018

  len =     944   nex =       0

>4895233        /34688

  len =    1882   nex =       4

   Term   57501   57086       -        0
   Intr   57848   57580       -        0
   Intr   58122   58008       -        0
   Init   58967   58722       -        0

>4914356        /19995

  len =    1154   nex =       2

   Term   25058   24628       -        0
   Init   25781   25678       -        0

>4914383        /41120

  len =    1665   nex =       4

   Init   69870   70209       +        0
   Intr   70434   70564       +        0
   Intr   70654   70875       +        0
   Term   70961   71525       +        0

>4914383        /22376

  len =    1122   nex =       5

   Init   93930   94034       +        0
   Intr   94116   94290       +        0
   Intr   94386   94491       +        0
   Intr   94572   94661       +        0
   Term   94741   95051       +        0

>4914399        /36778

  len =    2278   nex =       8

   Term   17584   17205       -        0
   Intr   17738   17692       -        0
   Intr   18153   18069       -        0
   Intr   18331   18253       -        0
```

09595326 .061600

```
   Intr   18578   18425        -          0
   Intr   18790   18711        -          0
   Intr   19120   18889        -          0
   Init   19482   19368        -          0

>4914399        /97049

  len =     537   nex =       1

   Sngl   61982   61446        -          0

>4914400        /20435

  len =    1768   nex =       7

   Term   20696   20292        -          0
   Intr   20940   20830        -          0
   Intr   21127   21041        -          0
   Intr   21289   21216        -          0
   Intr   21473   21380        -          0
   Intr   21619   21574        -          0
   Init   22059   21711        -          0

>4914400        /17491

  len =     491   nex =       1

   Sngl   25638   25148        -          0

>4914400        /32381

  len =     674   nex =       1

   Sngl   30579   31252        +          0

>4914400        /124761

  len =     709   nex =       1

   Sngl   52209   51501        -          0

>4914400        /117732

  len =    1754   nex =       5

   Term   85506   85180        -          0
   Intr   85754   85603        -          0
   Intr   86180   85944        -          0
   Intr   86678   86458        -          0
   Init   86933   86763        -          0

>4914400        /42211

  len =     630   nex =       1

   Sngl   90161   89532        -          0

>4914422        /30671

  len =     735   nex =       2
```

```
      Term   11060   10810         -         0
      Init   11544   11267         -         0

 >4914422        /41723

     len =      523   nex =        1

      Sngl   16294   16816         +         0

 >4914422        /15801

     len =     1274   nex =        4

      Init   40051   40158         +         0
      Intr   40255   40329         +         0
      Intr   40525   40659         +         0
      Term   41043   41301         +         0

 >4914422        /21466

     len =     2006   nex =        3

      Init   46972   47324         +         0
      Intr   47772   47952         +         0
      Term   48182   48359         +         0

 >4914422        /28549

     len =     1090   nex =        3

      Init   50748   50839         +         0
      Intr   51200   51370         +         0
      Term   51631   51834         +         0

 >4914422        /147351

     len =     1099   nex =        3

      Init   50774   50839         +         0
      Intr   51200   51370         +         0
      Term   51631   51859         +         0

 >4914422        /15569

     len =      268   nex =        1

      Sngl   56936   56669         -         0

 >4914422        /152675

     len =      463   nex =        1

      Sngl   57037   56575         -         0

 >4914422        /32703

     len =     1041   nex =        3

      Term   57059   56669         -         0
      Intr   57259   57112        -          0
      Init   57709   57543        -          0

>4914422        /97001

     len =     1020   nex =       1

      Sngl   57709   56690        -          0

>4914422        /13439

     len =     1019   nex =       2

      Term   57259   56691        -          0
      Init   57709   57543        -          0

>4914422        /27704

     len =      989   nex =       2

      Term   57259   57112        -          0
      Init   57709   57543        -          0

>4914422        /104060

     len =     1030   nex =       3

      Term   57059   56690        -          0
      Intr   57259   57112        -          0
      Init   57710   57543        -          0

>4914422        /32548

     len =     1050   nex =       2

      Term   57259   56665        -          0
      Init   57714   57543        -          0

>4914422        /4740

     len =     1222   nex =       2

      Init   69466   69678        +          0
      Term   69803   69921        +          0

>4914422        /14629

     len =      730   nex =       2

      Init   71747   71925        +          0
      Term   72026   72475        +          0

>4914422        /34593

     len =     1780   nex =       5

      Init   73912   74057        +          0
      Intr   74215   74328        +          0
      Intr   74412   74925        +          0
      Intr   75022   75175        +          0
```

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Reference No. 2750-942P

```
     Term   75262   75691         +        0

>4914449        /33307

     len =     1602   nex =        3

     Term    9666    9504         -        0
     Intr   10587   10510         -        0
     Init   10901   10672         -        0

>4914449        /2508

     len =     2620   nex =        6

     Term   32362   32080         -        0
     Intr   32797   32572         -        0
     Intr   33006   32910         -        0
     Intr   33341   33085         -        0
     Intr   33603   33434         -        0
     Init   34699   34297         -        0

>4914454        /20902

     len =     1779   nex =        4

     Init   14139   14543         +        0
     Intr   14915   15176         +        0
     Intr   15255   15322         +        0
     Term   15419   15917         +        0

>4914454        /7447

     len =     1873   nex =        2

     Term   16739   15950         -        0
     Init   17822   17623         -        0

>4914454        /19084

     len =      339   nex =        1

     Sngl   18789   19127         +        0

>4914454        /118265

     len =      391   nex =        1

     Sngl   33814   33429         -        0

>4914454        /11319

     len =     2254   nex =        7

     Init    4901    4989         +        0
     Intr    5232    5572         +        0
     Intr    5666    5761         +        0
     Intr    5846    5923         +        0
     Intr    6011    6187         +        0
     Intr    6602    6670         +        0
     Term    6755    7154         +        0
```

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```
>4928204        /40175

  len =     2530    nex =       10

   Term   16431    15960         -        0
   Intr   16690    16520         -        0
   Intr   16930    16820         -        0
   Intr   17119    17018         -        0
   Intr   17277    17215         -        0
   Intr   17440    17369         -        0
   Intr   17638    17537         -        0
   Intr   17812    17711         -        0
   Intr   17983    17911         -        0
   Init   18119    18067         -        0

>4938473        /95855

  len =      940    nex =        2

   Init   34311    34428         +        0
   Term   34806    35250         +        0

>4938473        /21878

  len =     2202    nex =        5

   Term   75630    75293         -        0
   Intr   75942    75702         -        0
   Intr   76292    76068         -        0
   Intr   76487    76363         -        0
   Init   77494    77121         -        0

>4938473        /2891

  len =     1229    nex =        1

   Sngl    9654    10033         +        0

>4938473        /125503

  len =      234    nex =        1

   Sngl    9689     9922         +        0

>4938493        /21702

  len =     4165    nex =       13

   Init   13805    13896         +        0
   Intr   14185    14254         +        0
   Intr   14586    14698         +        0
   Intr   14905    15001         +        0
   Intr   15177    15256         +        0
   Intr   15440    15634         +        0
   Intr   15720    15803         +        0
   Intr   15895    15955         +        0
   Intr   16076    16136         +        0
   Intr   16239    16332         +        0
   Intr   16404    16476         +        0
```

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```
    Intr   16614   16696         +        0
    Term   16814   17269         +        0

>4938493        /14713

   len =     2612   nex =        6

    Term   37708   37490         -        0
    Intr   37918   37806         -        0
    Intr   38169   38036         -        0
    Intr   38398   38290         -        0
    Intr   38544   38485         -        0
    Init   38752   38670         -        0

>4938493        /26276

   len =      310   nex =        1

    Sngl   80685   80993         +        0

>4972043        /33435

   len =      796   nex =        1

    Sngl   16528   17323         +        0

>4972043        /22677

   len =      674   nex =        1

    Sngl   18856   19529         +        0

>4972043        /92148

   len =      970   nex =        4

    Init   25133   25274         +        0
    Intr   25355   25571         +        0
    Intr   25661   25801         +        0
    Term   25910   26100         +        0

>4972043        /2545

   len =     2009   nex =        3

    Init    2771    3001         +        0
    Intr    3642    3942         +        0
    Term    4073    4779         +        0

>4972043        /41319

   len =     1930   nex =        3

    Term   35309   35007         -        0
    Intr   35674   35613         -        0
    Init   36929   36399         -        0

>4972043        /35742

   len =     1947   nex =        2
```

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```
         Init       49      578          +         0
         Term      771     1995          +         0

 >4972043        /38287

       len =     1172    nex =        1

         Sngl    62463    63634          +         0

 >4972043        /7471

       len =     1810    nex =        6

         Init    92440    92483          +         0
         Intr    92601    92697          +         0
         Intr    92948    93047          +         0
         Intr    93126    93211          +         0
         Intr    93299    93390          +         0
         Term    93561    93675          +         0

 >4972043        /4398

       len =     1797    nex =        5

         Init    92470    92697          +         0
         Intr    92948    93047          +         0
         Intr    93126    93211          +         0
         Intr    93299    93390          +         0
         Term    93561    93675          +         0

 >4972043        /93806

       len =     1701    nex =        5

         Init    92571    92697          +         0
         Intr    92948    93047          +         0
         Intr    93126    93211          +         0
         Intr    93299    93390          +         0
         Term    93561    93675          +         0

 >4972043        /119346

       len =      647    nex =        0

 >4972065        /38344

       len =     1428    nex =        3

         Term    31960    31284          -         0
         Intr    32312    32044          -         0
         Init    32711    32422          -         0

 >4972065        /34069

       len =     1213    nex =        3

         Init    47383    47581          +         0
         Intr    47679    47884          +         0
         Term    48236    48595          +         0
```

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```
>4972065       /10069

   len =     434   nex =       1

    Sngl   48305   48738        +        0

>4972065       /36741

   len =    1705   nex =       4

    Init   49180   49398        +        0
    Intr   49482   50010        +        0
    Intr   50089   50447        +        0
    Term   50541   50884        +        0

>4972065       /101714

   len =     294   nex =       1

    Sngl   52352   52059        -        0

>4972065       /35207

   len =    1280   nex =       4

    Term   51432   51078        -        0
    Intr   51652   51532        -        0
    Intr   51983   51743        -        0
    Init   52357   52061        -        0
>4972065       /126440

   len =     391   nex =       1

    Sngl   59542   59932        +        0

>4972077       /12759

   len =    2470   nex =       2

    Term   17826   17297        -        0
    Init   18688   17900        -        0

>4972077       /25532

   len =    2549   nex =       2

    Term   17826   17288        -        0
    Init   18688   17900        -        0

>4972077       /116606

   len =     490   nex =       1

    Sngl   58084   58567        +        0

>4972087       /38343

   len =     817   nex =       1
```

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```
                                          1586
     Sngl   23707   24523         +         0

  >4972087        /28506

    len =    1074   nex =        3

     Init   28873   29020         +         0
     Intr   29372   29517         +         0
     Term   29781   29946         +         0

  >4972087        /30708

    len =     941   nex =        1

     Sngl   30856   29916         -         0

  >4972087        /38500

    len =    2072   nex =        7

     Init   33934   34065         +         0
     Intr   34547   34684         +         0
     Intr   34962   34999         +         0
     Intr   35096   35146         +         0
     Intr   35230   35397         +         0
     Intr   35468   35580         +         0
     Term   35674   36005         +         0

  >4972087        /115901

    len =    1524   nex =        2

     Term    8097    8047         -         0
     Init    9570    9200         -         0

  >4996901        /2496

    len =    1366   nex =        3

     Init   15599   15705         +         0
     Intr   15871   15982         +         0
     Term   16118   16964         +         0

  >4996901        /35390

    len =    2432   nex =        5

     Term     872     613         -         0
     Intr    1483    1364         -         0
     Intr    1822    1718         -         0
     Intr    2438    2370         -         0
     Init    3044    2539         -         0

  >4996901        /15624

    len =    1960   nex =        7

     Init   55525   55641         +         0
     Intr   55761   55942         +         0
     Intr   56052   56159         +         0
```

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```
     Intr    56321    56410          +         0
     Intr    56669    56753          +         0
     Intr    57080    57174          +         0
     Term    57264    57484          +         0

>4996901        /11543

     len =     2590    nex =       10

     Init    78466    78573          +         0
     Intr    78960    79043          +         0
     Intr    79136    79248          +         0
     Intr    79343    79556          +         0
     Intr    79691    79764          +         0
     Intr    79859    79912          +         0
     Intr    80008    80115          +         0
     Intr    80204    80262          +         0
     Intr    80494    80704          +         0
     Term    80794    81047          +         0

>4996901        /97320

     len =      639    nex =        2

     Term    81166    80888          -         0
     Init    81526    81238          -         0

>4996901        /36526

     len =     1570    nex =        4

     Init    81684    81730          +         0
     Intr    81908    82053          +         0
     Intr    82294    82409          +         0
     Term    82501    83245          +         0

>4996901        /37444

     len =     1696    nex =        3

     Init    83424    83855          +         0
     Intr    84133    84410          +         0
     Term    84602    85119          +         0

>4996901        /30264

     len =     1299    nex =        2

     Term    91084    90551          -         0
     Init    91849    91557          -         0

>4996902        /27688

     len =      879    nex =        2

     Term    61399    61074          -         0
     Init    61655    61591          -         0

>4996902        /142223
```

```
    len =     285    nex =        1

      Sngl   83376   83092        -         0

>4996902        /11977

    len =    2719    nex =        5

      Term   84065   83542        -         0
      Intr   84858   84288        -         0
      Intr   85264   84965        -         0
      Intr   85618   85341        -         0
      Init   86260   85915        -         0

>4996903        /21835

    len =    2193    nex =        4

      Term   60521   59989        -         0
      Intr   60800   60607        -         0
      Intr   61272   60875        -         0
      Init   62181   61788        -         0

>5002514        /22413

    len =    3101    nex =       12

      Term   22954   22566        -         0
      Intr   23196   23047        -         0
      Intr   23432   23292        -         0
      Intr   23601   23514        -         0
      Intr   23740   23685        -         0
      Intr   23895   23820        -         0
      Intr   24045   23972        -         0
      Intr   24285   24127        -         0
      Intr   24518   24375        -         0
      Intr   24733   24601        -         0
      Intr   24951   24845        -         0
      Init   25666   25475        -         0

>5002514        /24475

    len =    1016    nex =        2

      Term   34112   33856        -         0
      Init   34871   34579        -         0

>5002514        /15906

    len =    4330    nex =       13

      Term   47968   47811        -         0
      Intr   48454   48334        -         0
      Intr   48638   48589        -         0
      Intr   48846   48733        -         0
      Intr   49060   48980        -         0
      Intr   49228   49147        -         0
      Intr   49372   49318        -         0
      Intr   49764   49534        -         0
      Intr   50625   49920        -         0
```

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```
     Intr   51051   50964          -        0
     Intr   51239   51133          -        0
     Intr   51447   51329          -        0
     Init   52137   51799          -        0

>5002514       /15314

    len =     1710   nex =        4

     Init   69964   70199          +        0
     Intr   70466   70639          +        0
     Intr   70727   70894          +        0
     Term   71131   71673          +        0

>5019262       /93927

    len =      983   nex =        2

     Term   59395   58861          -        0
     Init   59843   59533          -        0

>5019264       /110927

    len =      415   nex =        1

     Sngl    4369    4766          +        0

>5019264       /122052

    len =      370   nex =        1

     Sngl    4404    4766          +        0

>5019264       /23133

    len =      743   nex =        1

     Sngl   98229   97508          -        0

>5019265       /2686

    len =     1280   nex =        5

     Term      83       1          -        0
     Intr     243     167          -        0
     Intr     604     337          -        0
     Intr     923     718          -        0
     Init    1280    1010          -        0

>5041959       /117665

    len =      778   nex =        1

     Sngl   29026   29803          +        0

>5041960       /39877

    len =     1241   nex =        1

     Sngl   33119   34355          +        0
```

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```
>5041962        /32146

   len =    2478    nex =       7

     Init   27173   27393         +        0
     Intr   27737   27765         +        0
     Intr   28119   28208         +        0
     Intr   28281   28520         +        0
     Intr   28603   28800         +        0
     Intr   28954   29072         +        0
     Term   29160   29650         +        0

>5041962        /37936

   len =    1330    nex =       4

     Init   36213   36536         +        0
     Intr   36817   36933         +        0
     Intr   37029   37116         +        0
     Term   37380   37537         +        0

>5041962        /28093

   len =    1450    nex =       4

     Init   36215   36536         +        0
     Intr   36817   36933         +        0
     Intr   37029   37116         +        0
     Term   37380   37657         +        0

>5041962        /109138

   len =     692    nex =       2

     Init   36222   36536         +        0
     Term   36817   36901         +        0

>5041968        /92527

   len =    1055    nex =       2

     Term   39971   39857         -        0
     Init   40467   40315         -        0

>5051726        /5364

   len =    1619    nex =       3

     Init  106365  106639         +        0
     Intr  106745  106850         +        0
     Term  107554  107983         +        0

>5051726        /19178

   len =    1333    nex =       5

     Init   18384   18450         +        0
     Intr   18595   18622         +        0
     Intr   19014   19098         +        0
```

```
        Intr   19213   19302         +        0
        Term   19395   19716         +        0

    >5051726        /31050

        len =    1255   nex =        4

        Init   18401   18450         +        0
        Intr   19014   19098         +        0
        Intr   19213   19302         +        0
        Term   19395   19642         +        0

    >5051726        /5398

        len =     761   nex =        1

        Sngl    9718    8958         -        0
```

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CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which encodes an amino acid sequence exhibiting at least 40% sequence identity to an amino acid sequence encoded by

(a) a nucleotide sequence described in Table 1 or a fragment thereof; or

(b) a complement of a nucleotide sequence shown in Table 1 or a fragment thereof.

2. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which exhibits at least 65% sequence identity to

(a) a nucleotide sequence shown in Table 1 or a fragment thereof; or

(b) a complement of a nucleotide sequence described in Table 1 or a fragment thereof.

3. An isolated nucleic acid molecule comprising a nucleic acid having a nucleotide sequence which exhibits at least 65% sequence identity to a gene comprising

(a) a nucleotide sequence shown in Table 1 or a fragment thereof; or

(b) a complement of a nucleotide sequence described in Table 1 or a fragment thereof.

4. An isolated nucleic acid molecule which is the reverse of the isolated nucleotide sequence according to claim 1, such that the reverse nucleotide sequence has a sequence order which is the reverse of the sequence order of said isolated nucleotide sequence according to claim 1.

5. An isolated nucleic acid molecule comprising a nucleic acid capable of hybridizing to a nucleic acid having a sequence selected from the group consisting of:

(a) a nucleotide sequence which is shown in Table 1; and

(b) a nucleotide sequence which is complementary to a nucleotide sequence shown in Table 1;

under conditions that permit formation of a nucleic acid duplex at a temperature from about 40°C and 48°C below the melting temperature of the nucleic acid duplex.

6. The nucleic acid molecule according to claim 1, wherein said nucleic acid comprises an open reading frame.

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7. The isolated nucleic acid molecule of claim 1, wherein said nucleic acid is capable of functioning as a promoter, a 3' end termination sequence, an untranslated region (UTR), or as a regulatory sequence.

8. The isolated nucleic acid molecule of claim 7, wherein said nucleic acid is a promoter and comprises a sequence selected from the group consisting of a TATA box sequence, a CAAT box sequence, a motif of GCAATCG or any transcription-factor binding sequence, and any combination thereof.

9. The isolated nucleic acid molecule of claim 7, wherein the nucleic acid sequence is a regulatory sequence which is capable of promoting seed-specific expression, embryo-specific expression, ovule-specific expression, tapetum-specific expression or root-specific expression of a sequence or any combination thereof.

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10. A vector construct comprising a nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is heterologous to any element in said vector construct.

11. A vector construct comprising:

(a) a first nucleic acid having a regulatory sequence capable of causing transcription and/or translation; and

(b) a second nucleic acid having the sequence of the isolated nucleic acid molecule according to claim 1;

wherein said first and second nucleic acids are operably linked and
wherein said second nucleic acid is heterologous to any element in said vector construct.

12. The vector construct according to claim 11, wherein said first nucleic acid is native to said second nucleic acid.

13. The vector construct according to claim 11, wherein said first nucleic acid is heterologous to said second nucleic acid.

14. A vector construct comprising:

(c) a first nucleic acid having the sequence of the isolated nucleic acid molecule according to claim 7; and

(d) a second nucleic acid;

wherein said first and second nucleic acids are operably linked and
wherein said first nucleic acid is heterologous to any element in said vector construct.

15. The vector construct according to claim 14, wherein said first nucleic acid is native to said second nucleic acid.

16. The vector construct according to claim 14, wherein said first nucleic acid is heterologous to said second nucleic acid.

17. A host cell comprising an isolated nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is flanked by exogenous sequence.

18. A host cell comprising a vector construct of claim 10.

19. A host cell comprising a vector construct of claim 11.

20. A host cell comprising a vector construct of claim 12.

21. A host cell comprising a vector construct of claim 13.

22. A host cell comprising a vector construct of claim 14.

23. A host cell comprising a vector construct of claim 15.

24. A host cell comprising a vector construct of claim 16.

25. An isolated polypeptide comprising an amino acid sequence

(a) exhibiting at least 40% sequence identity of an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof; and

(b) capable of exhibiting at least one of the biological activities of the polypeptide encoded by said nucleotide sequence shown in Table 1 or a fragment thereof.

26. The isolated polypeptide of claim 25, wherein said amino acid sequence exhibits at least 75% sequence identity to an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof.

27. The isolated polypeptide of claim 25, wherein said amino acid sequence exhibits at least 85% sequence identity to an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof.

28. The isolated polypeptide of claim 25, wherein said amino acid sequence exhibits at least 90% sequence identity to an amino acid sequence encoded by a sequence shown in Table 1 or a fragment thereof.

29. An antibody capable of binding the isolated polypeptide of claim 25.

30. A method of introducing an isolated nucleic acid into a host cell comprising:
   (a) providing an isolated nucleic acid molecule according to claim 1; and
   (b) contacting said isolated nucleic with said host cell under conditions that permit insertion of said nucleic acid into said host cell.

31. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 10.

32. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 11.

33. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 12.

34. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 13.

35. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 14.

36. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 15.

37. A method of transforming a host cell which comprises contacting a host cell with a vector construct according to claim 16.

38. A method of modulating transcription and/or translation of a nucleic acid in a host cell comprising:

(a) providing the host cell of claim 17; and
(b) culturing said host cell under conditions that permit transcription or translation.

39. A method for detecting a nucleic acid in a sample which comprises:

(a) providing an isolated nucleic acid molecule according to claim 1;
(b) contacting said isolated nucleic acid molecule with a sample under conditions which permit a comparison of the sequence of said isolated nucleic acid molecule with the sequence of DNA in said sample; and
(c) analyzing the result of said comparison.

40. The method according to claim 39, wherein said isolated nucleic acid molecule and said sample are contacted under conditions which permit the formation of a duplex between complementary nucleic acid sequences.

41. A plant or cell of a plant which comprises a nucleic acid molecule according to claim 1 which is exogenous to said plant or plant cell.

42. A plant or cell of a plant which comprises a nucleic acid molecule according to claim 1, wherein said nucleic acid molecule is heterologous to said plant or said cell of a plant.

43. A plant or cell of a plant which has been transformed with a nucleic acid molecule according to claim 1.

44. A plant or cell of a plant which comprises a vector construct according to claim 10.

45. A plant or cell of a plant which has been transformed with a vector construct according to claim 10.

46. A plant which has been regenerated from a plant cell according to claim 41.

47. A plant which has been regenerated from a plant cell according to claim 42.

48. A plant which has been regenerated from a plant cell according to claim 43.

49. A plant which has been regenerated from a plant cell according to claim 44.

50. A plant which has been regenerated from a plant cell according to claim 45.

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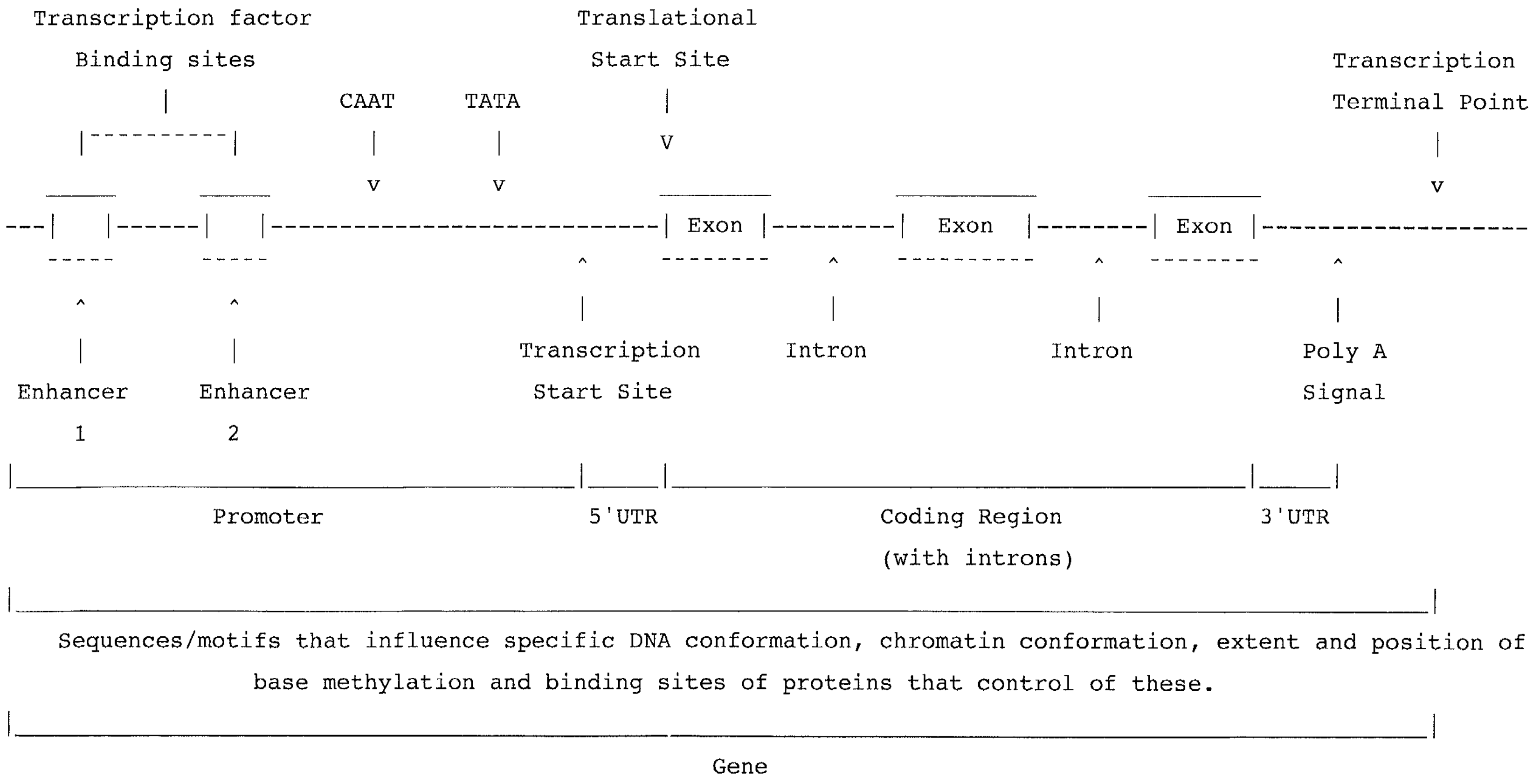
SCHEMATIC 1

SCHEMATIC OF A GENE



ABSTRACT OF THE DISCLOSURE

The present invention provides DNA molecules that constitute fragments of the genome of a plant, and polypeptides encoded thereby. The DNA molecules are useful for specifying a gene product in cells, either as a promoter or as a protein coding sequence or as an UTR or as a 3' termination sequence, and are also useful in controlling the behavior of a gene in the chromosome, in controlling the expression of a gene or as tools for genetic mapping, recognizing or isolating identical or related DNA fragments, or identification of a particular individual organism, or for clustering of a group of organisms with a common trait.

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POWER OF ATTORNEY

CERES, INC.
3007 Malibu Canyon Road
Malibu, CA 90265

I, Richard Hamilton, Chief Financial Officer of CERES, INC. of 3007 Malibu Canyon Road, Malibu, California 90265, grant Power of Attorney and authority to empower the following attorneys to act on behalf of CERES, INC. for executing Verified Statements (Declarations) Claiming Small Entity Status to be submitted to the U.S. Patent and Trademark Office in connection with the filing of provisional or regular patent applications on behalf of CERES, INC.

Raymond C. Stewart (Reg. No. 21,066)
Joseph A. Kolasch (Reg. No. 22,463)
Leonard R. Svensson (Reg. No. 30,330)
Gerald M. Murphy, Jr. (Reg. No.28,977)
Mark J. Nuell (Reg. No. 36,623)

This Power of Attorney is to remain in full force and effect until terminated by an official of CERES, INC.

By______________________________
Richard Hamilton

Date 9/24/98